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A COMPARISON OF UTERINE WEIGHTS AND LENGTHS IN VIRGIN AND POSTPARTUM RATS[1]

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Received March 21, 1960

The purpose of this paper is to give information about the lengths and weights of uteri from virgin and uniparous rats during different stages of the estrous cycle, and from immature and pseudopregnant animals. Osterud (2) reported that uteri from mature rats weighed more than those from immature animals. He also found that involuted postpartum uteri weighed much less than those from virgin animals. Souders and Morgan (3) obtained essentially similar results in post-lactation animals, although they noted that the weight of uteri one day after parturition was greater than that of uteri from virgin animals. They did not indicate the stage of the estrous cycle at which the involuted postpartum uteri were taken. Astwood (1) found that uterine weight is maximal at proestrus and minimal on the first day of diestrus. On pseudopregnant rats the uterine weight regresses below the diestrus level after the fifth day.

MATERIALS AND METHODS

Albino rats of the Wistar strain whose previous history was known were used in this study. Immature animals were six to seven weeks old when sacrificed. Virgin, pseudopregnant and primiparous rats were four to five months old at sacrifice. Vaginal smears were examined to determine stages of the estrous cycle.

Pseudopregnant rats were obtained by mating estrous females with a vasectomized male. Females showing a vaginal plug the following morning were removed. If these animals continued to exhibit a diestrus smear continuously for five days they were considered as pseudopregnant. The females thus obtained were sacrificed on the sixth day of pseudopregnancy; the first day being counted from the day prior to the finding of the diestrus type of smear.

[1] Supported by USPH Grant R. G. 5102.

Primiparous rats were obtained by placing young virgin females in breeding cages with mature males in the ratio of five to one. From time to time the visibly pregnant females were removed and each one was placed in a separate cage. They were allowed to go to term and the date of birth of each of the litters was recorded. The young were weaned at twenty-three to twenty-four days of age. Between twenty and twenty-five days after the termination of lactation, those females having uniform estrous cycles were sacrificed at the required stage.

The rats were weighed before being sacrificed. The reproductive tract was cut at cervix and released from the surrounding tissue. It was then lifted and the uterine cornua were pinned to a board covered with a cold wet paper towel. Extreme care was taken not to over stretch the uterine horns. To check this, the uterine horns of some were measured 'in situ' and remeasured after removal from the rat. The mesometrium was then cut as close to the uterus as possible. The length of the uterine horns from the tubo-uterine junction to the anterior end of the cervix was taken by a metric rule. The uterine horns were cut and weighed on a Roller-Smith torsion balance and the weights recorded. All data about uterine weight are analyzed and presented as relative weights, *i.e.*, milligrams of uterus per 100 grams of body weight.

Results

A. *Length of the Uterine Horns*

Measurements of the total lengths of the uterine horns of immature, virgin, pseudopregnant, and primiparous rats indicate that the right horns are significantly longer (P less than .05) than the left horns in all groups except the immature rats (Fig. 1). The mean length of both cornua was found to be greatest in virgin and postpartum rats at diestrus. Pseudopregnant rats have almost the same mean uterine length as that of the postpartum diestrus group. A comparison of the total uterine lengths (sum of right and left horns) of the different groups showed that the uterine length in the pseudopregnant and postpartum rats is more than that of the immature and virgin rats (P less than .005).

B. *Weights of the Uterine Horns*

Data about weights of the uterine horns (Fig. 2) indicate that the mean weights of the right horns are greater than those of corresponding

left horns in all virgin, pseudopregnant and postpartum groups (P less than .05). There is no significant difference between the mean weights of the right and left horns in immature rats (P greater than .05).

The uteri taken from postpartum rats at any stage of the estrous cycle are significantly heavier (P less than .05) than those from virgin rats at corresponding stages of the estrous cycle. Uteri from virgin rats during estrus and proestrus weigh more than those from immature rats (P less than .005 and .025 respectively). During diestrus, however, the virgin uterus is not significantly heavier than the immature uterus (P greater than .05). Within both the virgin and primiparous groups the uterus at diestrus weighs less than at either estrus or proestrus (P less than .05). The pseudopregnant uterus weighs significantly less than the postpartum diestrus, proestrus or estrous uterus (P less than .05, .0025, and .0025 respectively).

Discussion

The data presented in this study show that in the rat the right uterine horn is longer than the left horn. This asymmetry is particularly marked in the majority of the mature animals. The length of the uterus of primiparous rats does not regress to that of virgin animals even three to four weeks after the termination of lactation.

The report of Astwood (1) that the uterine weight is minimal at diestrus is confirmed. The observations of Osterud (2) and Souders and Morgan (3) that uteri of mated (postpartum) rats three weeks after the end of the first lactation weigh less than those of unmated (virgin) animals could not be confirmed in this study. However the data obtained show that the relative uterine weight of the virgin proestrus rat is more than that of the uterus of the primiparous diestrus rat three to four weeks following the termination of lactation. It is possible that Souders and Morgan (3) killed their animals at these two stages of the estrous cycle. Apparently the unmated controls were killed at early proestrus or estrus but they do not indicate the stage at which the postpartum animals were killed. If a random sample of postpartum animals were killed, the majority of them would be in diestrus since in a typical estrous cycle the diestrus stage is about twice the length of the estrous and proestrous period. In such a case the observation of Souders and Morgan (3) would be expected.

FIGURE 1

Length of uterine horns during stages of the estrous cycle in immature, virgin, and primiparous rats. One half of each cross-hatched bar plus the white bar at either end denotes one standard deviation on either side of the mean. The cross-hatched portion comprises two standard errors on either side of the mean. The middle vertical line shows the range with the figure at the upper end being the sample size and the letter at the lower end indicating either right or left horn.

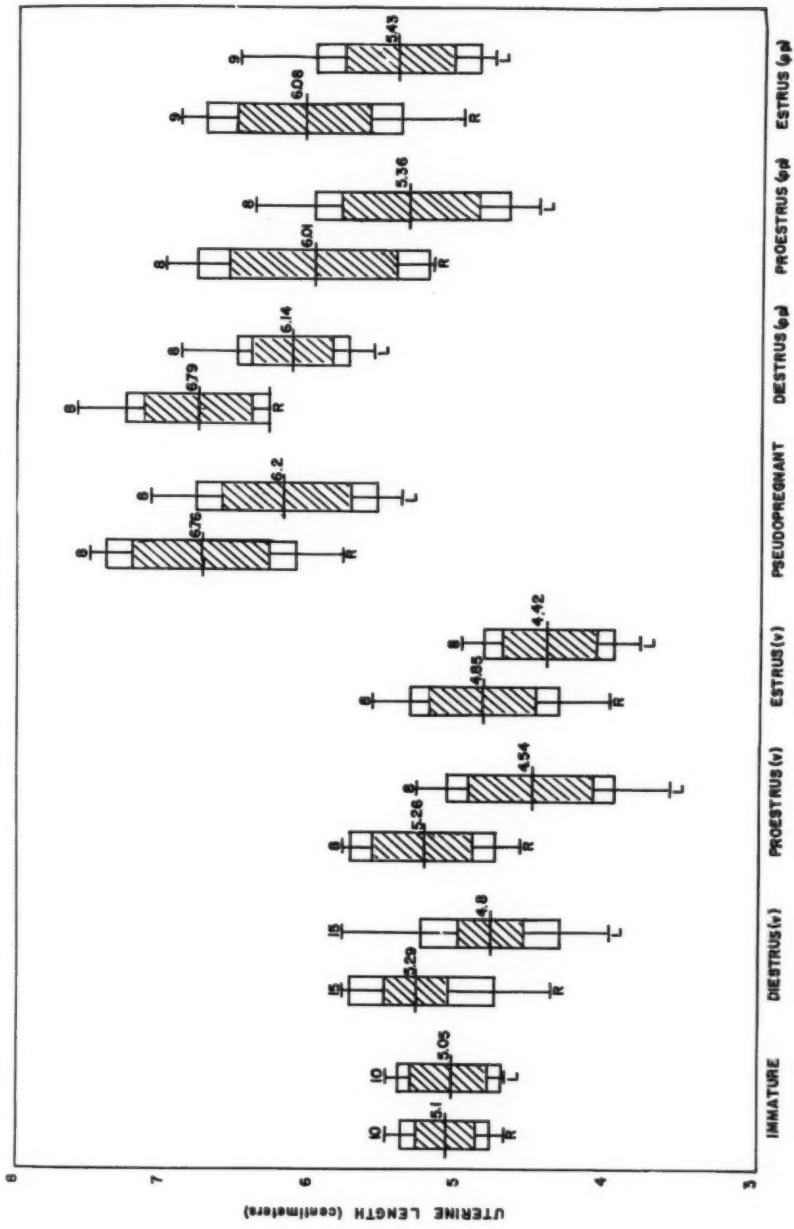
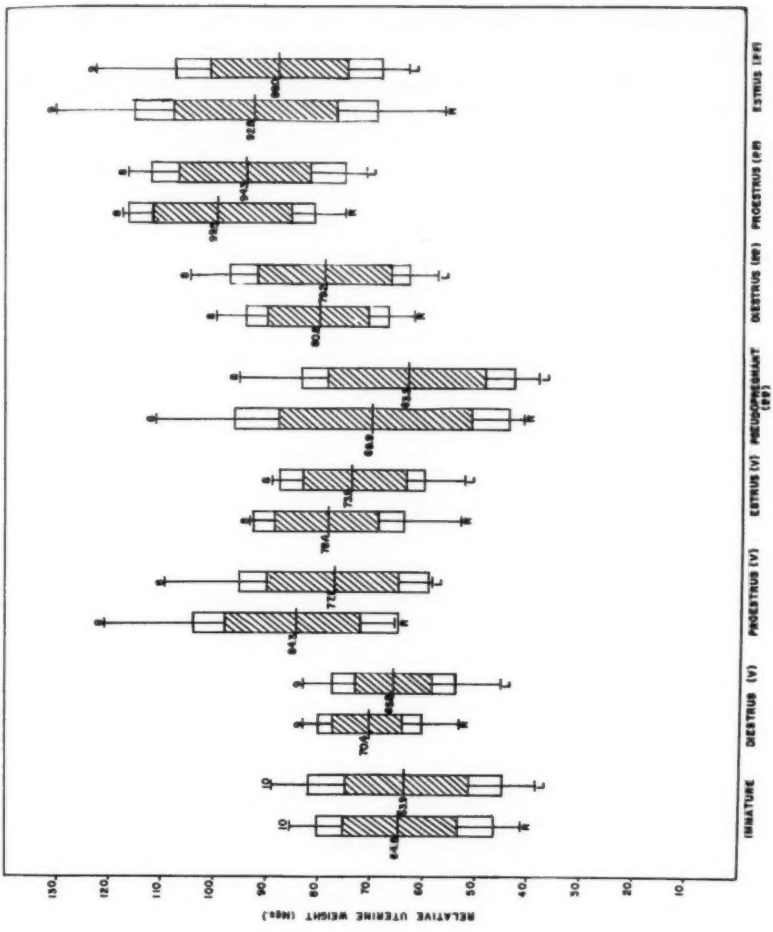

UTERINE LENGTH (centimeters)
8
7
6
5
4
3
IMMATURE
DIESTRUS (v)
PROESTRUS (v)
ESTRUS (v)
PSEUDOPREGNANT
DIESTRUS (φ)
PROESTRUS (φ)
ESTRUS (φ)
10
5.1
L
10
5.05
R
15
5.29
L
15
4.8
R
8
5.26
L
8
4.54
R
8
4.85
L
8
4.42
R
8
6.76
L
8
6.2
R
8
6.79
L
8
5.14
R
8
6.01
L
8
5.36
R
9
6.08
L
9
5.43
R

FIGURE 2

Weights of uterine horns during stages of the estrous cycle in immature, virgin and primiparous rats. All weights are expressed as milligrams of uterine tissue per 100 grams of body weight. Symbols are as in Figure 1.


RELATIVE UTERINE WEIGHT (MG%)
150
120
110
100
90
80
70
60
50
40
30
20
10
IMMATURE
DIESTRUS (V)
PROESTRUS (V)
ESTRUS (V)
PSEUDOPREGNANT (PP)
DIESTRUS (PP)
PROESTRUS (PP)
ESTRUS (PP)

Summary

1. Comparative data about the lengths and weights of the uteri of immature, virgin, pseudopregnant and primiparous rats are presented in this study.

2. The right uterine horn was longer and heavier than the left in all groups except the immature rats.

3. The means of absolute and relative weights of uteri from primiparous animals were greater than those of virgin animals at comparable stages of the estrous cycle.

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STERILE CULTURE OF THE SHOOT APEX OF *LUPINUS ALBUS**

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Received April 4, 1960

The growth of an angiospermous plant shoot from the shoot apex alone (the dome of meristematic tissue above the youngest recognizable foliar primordium) represents a goal toward which considerable effort has been directed. Ball (1946) demonstrated that in *Lupinus albus* as well as in *Tropaeolum majus* an entire plant could be grown from the shoot apices plus the three youngest visible foliar primordia and a small amount of subjacent tissue—a total volume of $430\mu^3$. Experiments at that time, and during the subsequent time, by this investigator have repeatedly confirmed the failure of the shoot apex alone of these plants to grow into a shoot in sterile culture upon a simple medium containing minerals, sugar, water and agar. The suggestion may be made that the angiospermous shoot apex has achieved a biochemical differentiation of such nature that it requires certain accessory substances that may be produced by young leaves or by subjacent tissues. Such biochemical differentiation may be considered an advanced state of evolution in comparison with that in lower vascular plants such as club mosses, horsetails and ferns, where the shoot apex, or a portion thereof, will grow in sterile culture (Wetmore, 1954) to produce a whole plant. The amino acids of the shoot apex of *Lupinus albus* were measured quantitatively by Steward, Wetmore, Thompson and Nitsch (1954). It was found that these compounds in a culture medium had no growth-promoting value to the shoot apex of this plant; interestingly, however, such a combination of amino acids did accelerate the growth of shoot apices of *Adiantum* (Wetmore, 1954).

The present study was undertaken to determine whether certain recent information on the growth of maturing plant tissues might be of value in making a culture medium that would permit the growth

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of the shoot apex of *Lupinus albus*. If such growth were achieved, it was considered further of interest to note the effects of the important accessory substances on the maturing subjacent tissues in comparison to those upon the shoot apex. The shoot apex being an indeterminate meristem, a truly effective culture medium must contain those substances that will permit it to grow continuously. In other words, the hypothetical "adequate" culture medium would permit the shoot apex to produce not only new leaves and subjacent tissues of the primary shoot, but also to replace itself and to maintain itself in the "meristematic state," or as an indeterminate meristem. Any contribution to this problem would be of great fundamental importance, for it would give us some inkling of how, biochemically, the "open system of growth" of the angiospermous vegetative shoot is maintained.

Materials and Methods

White lupine plants between 15 and 30 days old were used. Shoot apices (the dome of meristematic tissue above the youngest recognizable foliar primordium, as shown in Fig. 1) were excised by utilizing sterilized microscalpels which had been made according to the directions given by Ball (1946). The piece of tissue, or inoculum, excised was approximately 300μ in each of the dimensions of width and of thickness and about 100μ in height. Great care was taken to exclude foliar primordia in the excisions and the inoculum consisted only of the dome of meristematic tissue (Fig. 2).

The combination of minerals suggested by Heller (1953) was used with the microelements listed by Ball (1946). Glucose was found to support better growth than sucrose, and was used, accordingly, in all experiments reported here. The optimum concentration of this hexose was found to be 7.5 g./1. of medium. The other concentrations tried, 3.8, 15, or 30 g./1., give inferior growth. Amino acids and other putative growth-supporting compounds, as well as sugar and minerals, were sterilized by filtration through fritted glass filters and added to the agar-water portion of the medium that had been previously sterilized by autoclaving and had been allowed to cool, prior to solidification, almost to room temperature. Each culture tube was vigorously rotated to insure thorough mixing of all components before the agar was allowed to solidify. All excisions of apices and inoculations were performed in a sterile transfer chamber

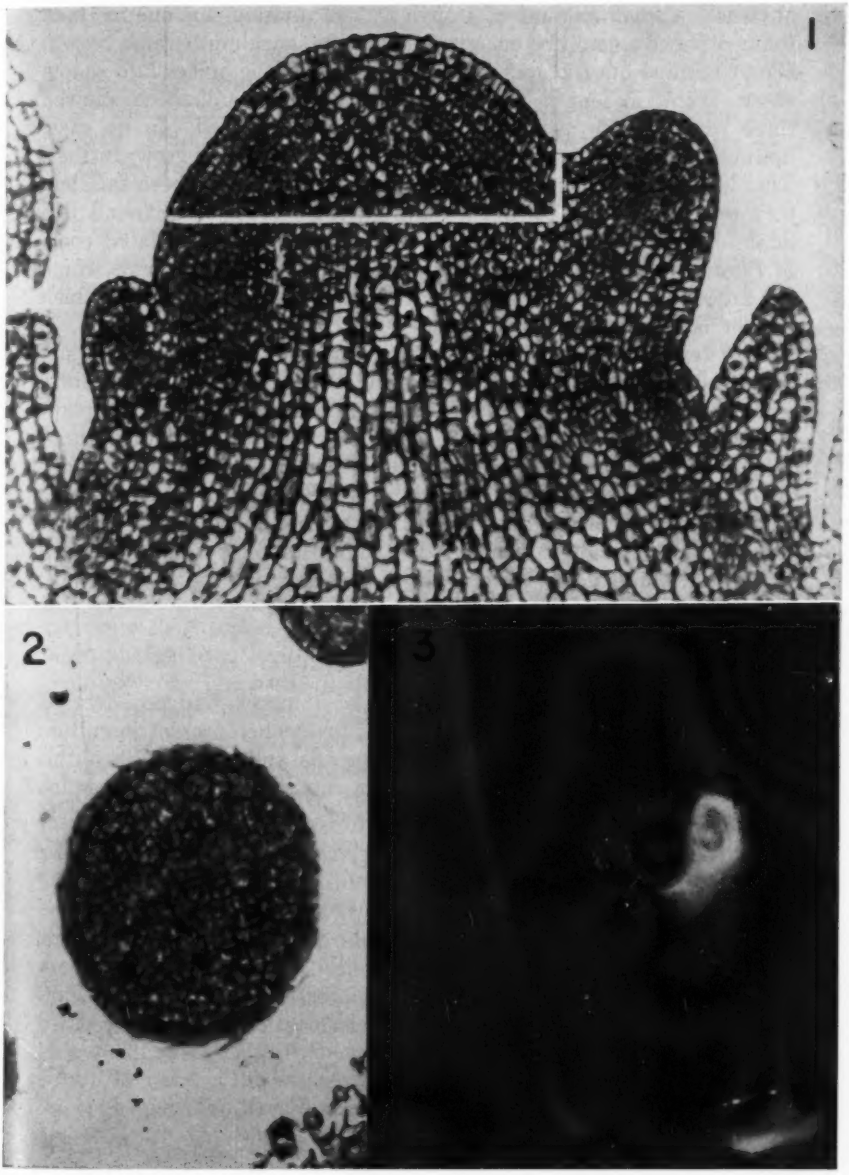
without flaming of culture tube mouths. Fifty apices were excised for each trial of a medium and inoculated upon agar slants. Each culture tube contained 20 ml. of medium. All cultures were maintained in the dark at 21C° in an air-conditioned culture room. Each shoot apex was manipulated, after excision, onto the tip of the flat side of the microscalpel. Inoculation consisted in pulling the opposite side of the knife over the surface of the slant until the shoot apex was pulled off and rested, tip outward, on the agar medium. A small volume of water always accumulated at the bottom of the slant; the apex was always carefully placed at least 1 cm. above the miniscus.

Effects of Amino Acids

An artificial combination of many amino acids that has been shown to support the formation and growth of "adventive embryos" in callus cultures of carrot (Reinert, 1959) did not support the growth of the shoot apex of *Lupinus*. Naturally-occurring combinations of amino acids in hydrolyzed soybean (enzymatically hydrolyzed), casein (HCl-hydrolyzed) at 0.1 g./1. or at 1 g./1. of culture medium permitted growth of the shoot apices to the extent of producing shoots about 5 mm. in length. Coconut milk (which is a combination of amino acids and other substances) at concentrations of 50, 100, or 150 ml./1. permitted somewhat better growth and shoots grew to a maximum of 1 cm. Nine amino acids (each of which normally occurs in the tissues of the shoot apex of this plant) were utilized singly in the culture medium at concentrations of 0.00006, 0.00012, 0.00025 or 0.0005 M: l-glutamic, l-alanine, l-arginine, l-methionine, l-phenylalanine, l-tryptophane, gamma-amino-butyric acid, l-glutamine, l-asparagine. These amino acids occurred in the non-protein nitrogen of the shoot apex of this plant (see table 2 of Steward et al., 1954) in percentages of the dry weight varying from 1.4 for tryptophane to 65.5 for aspargine. There appeared to be no significance in the percentage of occurrence of an amino acid in the shoot apex for its utilizability as a substrate. All of these amino acids, except gamma-amino-butyric acid, showed apparent toxicity to the tissues of the shoot apex and there was little or no growth. In examinations of the cultured apices under the binocular dissecting microscope at periods of time up to two months after inoculation, the conclusion "no growth" necessarily included some variation in appearance. In some cases, the apices turned brown without any change in shape. In others, there was

FIGURES 1, 2 AND 3

Fig. 1. Longitudinal section of a stem tip taken from a thirty-day-old plant. The shoot apex (the dome of embryonic tissue above the level of the youngest foliar primordium) is here separated by a cut to indicate the amount of tissue included in an excision which constituted an inoculum. Groups of cells are included within a common wall. (330×.) Fig. 2. Transverse section of a shoot apex slightly above the level of the excising cuts. The groups of cells within a common wall are evident throughout the dome of meristem. (330×.) Fig. 3. Shoot grown upon medium containing 0.00006 M gamma-amino-butyric acid. A shoot ca. 1.5 cm in length was produced here, terminating in a bud consisting of 5 foliar primordia around the shoot apex. The basal tissue was callus-like, dark in color, and functioned as the absorbing region of the shoot. Since this shoot attained only a small stature and light weight, it remained in its original position on the agar slant above the meniscus of liquid near the bottom of the tube. (2×.)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obviously a small amount of growth by cell division, for one to three foliar primordia could be observed before the inoculum became brown. Gamma-amino-butyric acid permitted growth of the apices into shoots about 1 cm. in length (Fig. 3). Microscopic examination showed three to five foliar primordia to have been produced. In no case, however, did the leaves or the subjacent stem tissue grow further. This limited growth may be contrasted with the much more satisfactory results obtained by Barnes and Naylor (1959) who found this amino acid to be among the best nitrogen sources for isolated roots of *Pinus serotina*. In addition to the evaluation of amino acids which are known to occur in the tissues of the shoot apex, two others which do not occur there, l-cystine and l-cysteine, were also tested. No growth resulted. With regard to the amino acids other than gamma-amino-butyric acid, the results of the present investigation differ somewhat from those of other workers who used various other plant tissues or organs. Fries (1953) found that the pea root was stimulated by 0.0003 M of arginie, glycine, and to a lesser extent, by glutamic acid. Fries' later work (1954) on the decotylized pea seedling showed no stimulation of growth, and often inhibition, by each of eighteen amino acids. Harris (1953) found inhibitory effects of each of eleven amino acids on growth of seedling oat roots; only one, l-arginine, appeared to be slightly stimulatory.

Effects of Growth Regulators

Indoleacetic acid at a concentration of 1 mg./l. had no effect on the growth of the shoot apex. 2,4-D was toxic when present in culture medium that contained only sugar, minerals and water. When this growth regulator was present in medium containing any of the amino acids tested, the toxicity was greatly increased. When present in medium with coconut milk, the shoot apex grew rapidly as a callus.

Effects of Vitamins

Biotin, vitamins B_1, B_6, B_{12} and C, folic acid, calcium pantothenate, p-aminobenzoic acid, riboflavin-5-phosphate, choline chloride, adenosine diphosphate-Na, pyridoxal-5-phosphate-H_2O, were tested individually in medium at appropriate concentrations. Generally, they appeared to be without effect on growth.

Effects of Derivatives of Nucleic Acid

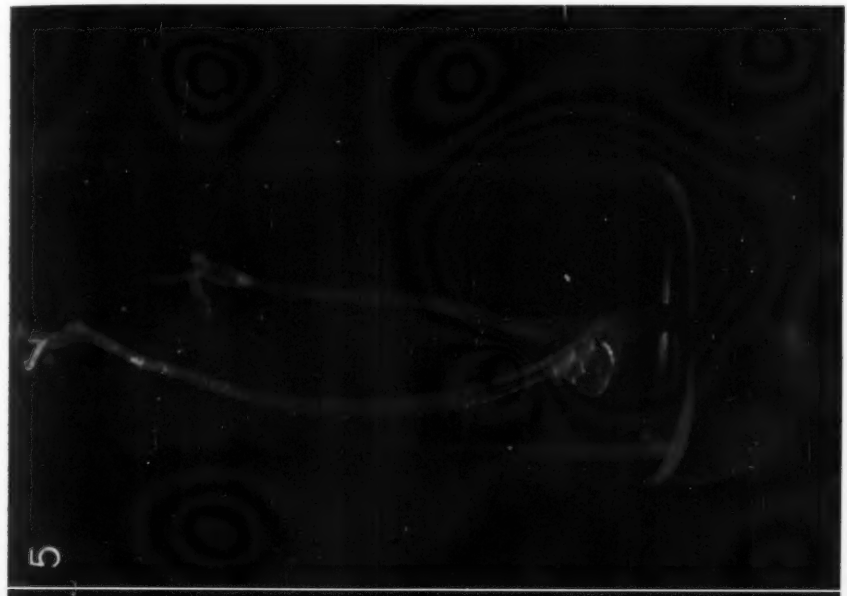
Starting with kinetin at 1 mg./1., adenine sulfate (1 to 132 mg./1.), xanthine, hypoxanthine, guanine-HCl, uric acid (each at 25 mg./1.) were tried. Kinetin had no effect, adenine was toxic, and moderate growth (shoots ca. 2 cm. in length) resulted when the others were in the medium.

Effects of Gibberellins

These growth factors, gibberellin or gibberellic acid, when present in the culture medium at 1, 2, 4, 8, 16 or 32 mg./l., appeared to bring about a persistence of the tissues of the shoot apex in the living condition. While growth was very little, the tissues remained in the living, green condition, as evidenced by lack of browning, when the cultures were discarded after five months. When the same concentration series of these growth factors was utilized in culture medium that also contained 150 ml./l. of coconut milk the shoot apices grew during the two-month period into shoots 5 to 10 cm in length (Fig. 4, 5) and produced seven to nine leaves (Fig. 73. It must be assumed that the gibberellin stimulated cell divisions within the shoot apex and in subjacent tissues, and increased the elongation of the subjacent maturing tissues. There was, however, no resumption of growth when the shoots were transferred to new culture medium of the same constitution. There was a strict distinction between a mass of basal tissue that was often brown on the surface, and a shoot that grew upward. The basal tissue was callus-like, while the stem, leaves, and shoot apex of the plantlet appeared to be normal (Fig. 6, 7). Close examination of the shoot apex of these cultured shoots, however, revealed that it was of considerably greater volume than the dome of a normal, soil-grown shoot (cf. Fig. 1). This enlargement appeared to derive from the increase in size of individual cells. Concomitant with the increase in cell-size, there was modification of the layering and the frequency of cell divisions. Evidence for the latter was the occurrence of relatively few derivative cells within one original cell wall (Fig. 8). This is known to be the normal mode of cell division within the shoot apex (Ball, 1949); a careful perusal of Figs. 1 and 2 shows large groups of cells within a surrounding wall. Fig. 8 shows a relatively early stage in this nutritional derangement of the tissues of a shoot apex. Fig. 9 shows a late stage in the process; not only do all the criteria of derangement listed above exist, but, in addition, the cells appear to have become highly vacuolate and lightly-staining.

FIGURES 4 AND 5

Fig. 4. A shoot grown on medium containing 150 ml./l. of coconut milk and 1 mg./l. of gibberellin. Leaflets of the longest leaf had not unfolded. The dividing line between the basal absorbing tissues and the shoot above them is sharp. (2×.) Fig. 5. A shoot grown on medium containing 150 ml./l. of coconut milk and 2 mg./l. of gibberellin. Leaflets of the longest leaves had begun to unfold. The size and weight of the shoot had caused it to fall off the original position upon the agar slant so that the basal tissues were in the liquid in the bottom of the tube. The liquid surrounding the basal tissues had, apparently, prevented their normal darkening. (2×.)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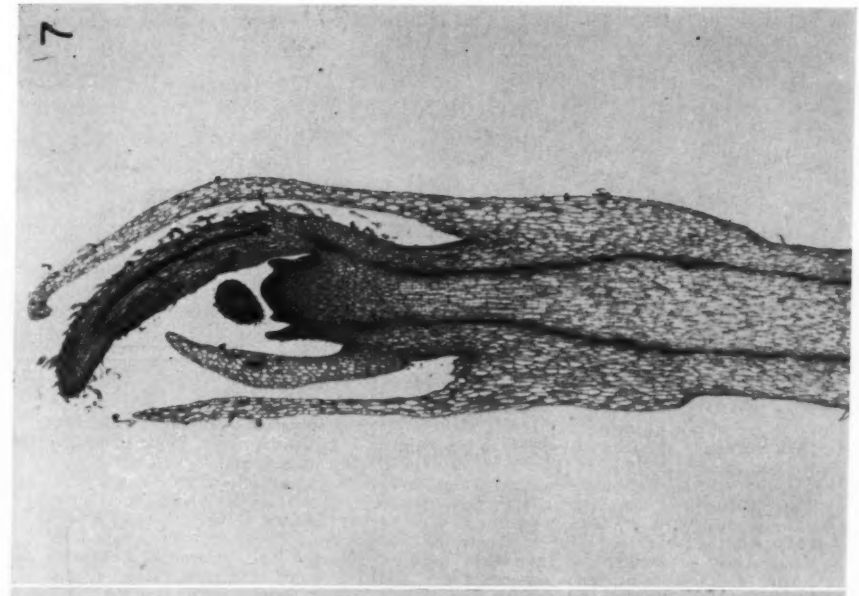
FIGURES 6 AND 7

Fig. 6. Median longitudinal section of a shoot grown on medium containing 150 ml./l. of coconut milk and 32 mg./l. of gibberellin. The sharp dividing line between the basal callus and the normal shoot above it is evident. The basal callus appears to have grown by divisions at its margins, and thus was not produced as part of a shoot by the shoot apex. The strands of vascular-like tissue were probably differentiated under the influence of the shoot apex. (21×.) Fig. 7. Median longitudinal section of the terminal portion of a shoot grown on medium containing 150 ml./l. of coconut milk and 1 mg./l. of gibberellin. In this shoot, as well as in that of Fig. 6, the shoot apex appears to be essentially normal as seen at this low magnification. (21×.)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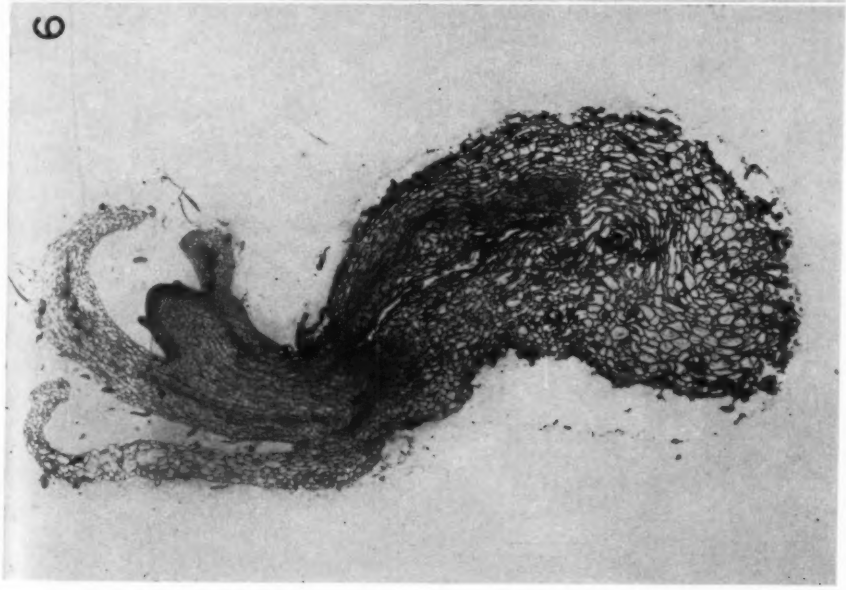
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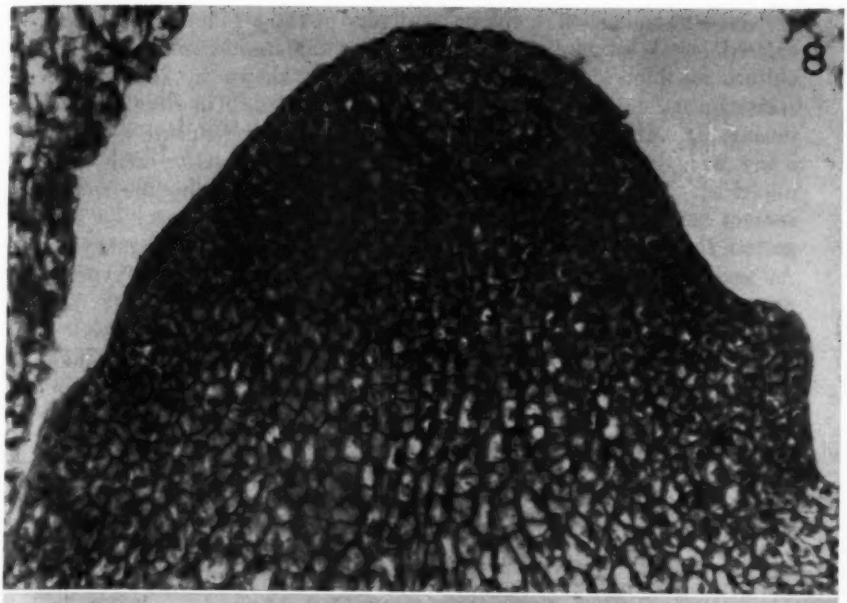
FIGURES 8 AND 9

Fig. 8. Median longitudinal section of the shoot apex of a plant grown for two months on medium containing 150 ml./l. of coconut milk and 1 mg./l. of gibberellin. The entire shoot apex is larger than normal, its cells are enlarged, and the normal layered condition of the cells has been lost. These changes have been interpreted as indications of the loss of meristematic qualities. (330×.) Fig. 9. Median longitudinal section of the shoot apex of a plant grown for two months on medium containing 150 ml./l. of coconut milk and 2 mg./l. of gibberellin. This shoot apex shows more pronounced indications of the loss of meristematic qualities: enlarged cells, enlarged condition of the entire embryonic region, pronounced vacuolation in all cells with the result that staining is faint, and development of intercellular spaces between cells. (330×.)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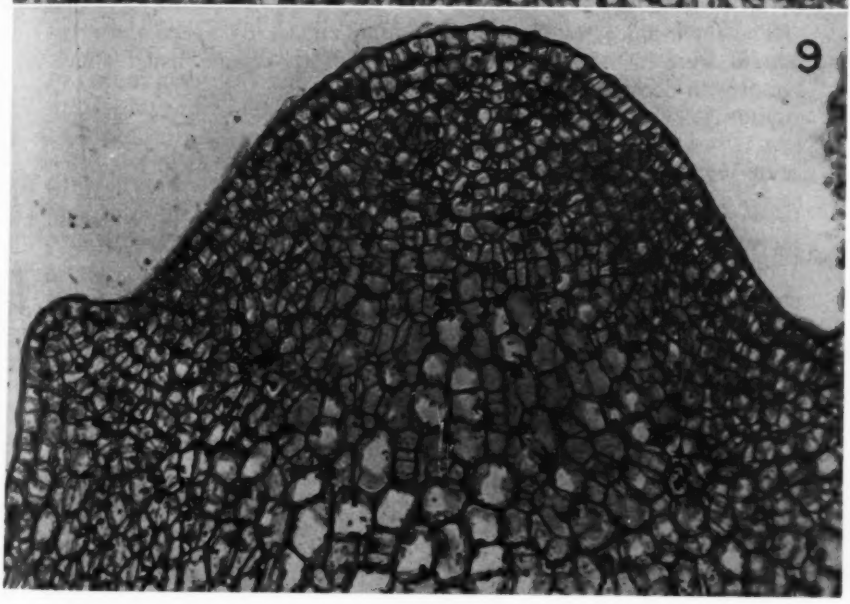
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A close examination of the basal tissues of these shoots grown from excised shoot apices revealed an entirely different response to the culture medium (Fig. 3, 4, 5, 6) from that shown by cells of the shoot apices and their later subjacent derivatives. These cells were, during the entire culture period, on contact with the culture medium. They were, furthermore, apparently the first derivative cells produced by the shoot apex; they were callus produced by the wound surface that lay in contact with the agar medium. It may be suggested that they became almost immediately mature parenchyma. As such, they appear to have undergone quite a different type of growth and differentiation from the normal stem tissues that were produced above them by the shoot apex. These subjacent tissues (Fig. 3, 4, 5) were obviously the absorbing-surface for the shoot. Their internal differentiation (Fig. 6) was so strikingly different from that of the normal shoot above them that it may be suggested that they grew and differentiated as independent callus, modified only by the presence of a normal bud attached above them which may have determined the differentiation of the subjacent abnormal strands of vascular-like tissue. No roots were ever formed from the basal tissues of any of the shoots produced from the shoot apex on any of the culture media utilized.

The shoot apices of *Tropaeolum majus* and of *Lycopersicum esculentum* were also inoculated upon each of the media listed above. No growth occurred in these apices.

Discussion

The fundamental differences in nutritional requirements of the tissues of the shoot apex, a primordial meristem, from those of subjacent tissues are well demonstrated by results of these experiments. Working with the shoot apex, three youngest foliar primordia, and a small amount of basal tissue, Ball (1946) demonstrated that such a bud could grow into an entire plant with adventitious roots. Coconut milk was shown at that time to have no effect upon the growth; it was suggested then that such an excision either contained, or was above to produce, all the accessory substances required for such growth. The present work, however, demonstrated that coconut milk has some effect, i.e., that it permits the tissues of the shoot apex to stay alive for a long period of time without much growth. The present work

further demonstrated that the shoot apex was probably self-sufficient for auxin, a number of vitamins, and several derivatives of nucleic acid.

The addition of gibberellin to the culture medium that contained coconut milk permitted the tissues of the shoot apex to go through a brief period of approximately normal growth in which several leaves and varying lengths of subjacent stem were produced. Since the inoculum included neither foliar primordia nor subjacent stem tissues, these results can be considered evidence that the shoot apex can produce these structures *in vitro* if given appropriate culture medium. The shoot apex was apparently normal during this period of growth, although near the end of two months its cells enlarged, lost their layered condition, and divided far less frequently so that comparatively few derivative cells appeared within the original wall of an initial cell. It is suggested that the culture medium did not contain certain essential factors for the continued growth of this primordial meristem. The combination of coconut milk and gibberellin provided a medium that could support only a limited period of normal growth, after which the tissues of the shoot apex underwent the first stages in maturation. The gibberellin can thus be regarded as a growth factor supporting cell divisions in the meristematic tissues of the shoot apex. The stimulatory effect of gibberellin on cell division in older tissues has been previously noted by Dure and Jensen (1957, in axes of cotton embryos) and by Sachs, Bretz and Lang (1959) in shoots of *Hyoscyamus* and *Samolus*. The conclusion of the latter authors that the stimulation of cell division in the shoot of *Hyoscyamus* was restricted to the subapical region and did not involve the shoot apex, is indeed surprising, since they noted that the treated plants produced many more leaves than the controls. In contrast, they showed that the shoot apices of *Samolus* were stimulated by gibberellin to undergo a two-fold increase in mitoses. These observations on both *Hyoscyamus* and *Samolus* are subject to question, since it is conceivable that the effects of subjacent tissues on shoot apex, and vice versa, had made the experiments extremely complex. Although no measurements of numbers of mitoses were made in the present work, it may be concluded by other growth observations that cell divisions were increased in the shoot apices as a result of addition of gibberellins to the culture medium. In controls, the number of foliar primordia on the cultured apices varied from none to three. In those experiments with gibber-

ellins in the culture medium, they varied from seven to nine. Cell divisions are necessary for the initiations of foliar primordia in all shoot apices (see Foster, 1936). Hence it must be concluded that the isolated shoot apex of *Lupinus albus* was stimulated by gibberellins to undergo more cell divisions than the controls.

Since the coconut milk-gibberellin medium would permit normal growth of the shoot apex for only a limited time, and such growth could not be prolonged or renewed by transfer to new medium, it may be tentatively concluded that other biochemical factors than those present in the tissues or the medium are essential. The necessity of much further research is indicated. This research could profitably take two directions. First, all known chemical growth factors must be tested for activity with the shoot apex of an angiosperm as a bioassay. Second, those shoot apices of lower vascular plants, e.g., ferns, horsetails, club-mosses, which are known to have the potentiality of growth in culture to produce an entire plant, must be more carefully studied. Extracts must be made from these shoot apices, and tested as substrata for the shoot apex of an angiosperm.

The present results can be considered to support the theory that the shoot apex of an angiosperm has undergone biochemical differentiation in the direction of having lost the ability to produce certain substances essential to its growth and maintenance as an indeterminate meristem. Comparatively, this deficiency appears to be extreme, if one judges from the biochemical substances necessary for the continued culture of a wide variety of angiospermous tissues (see Gautheret, 1959), none of which, however, was derived from a shoot apex. Researches such as the present one can thus possibly uncover some aspects of biochemical nutrition of plant tissues which could never be apparent from studies on tissues from mature or maturing parts of the plant.

Of particular significance in the present work is the finding that the cultured shoot apex, having completed a certain limited period of growth, not only ceases growth, but loses the cytological and histological characteristics of a meristem. This behavior differs fundamentally from that of a shoot apex upon an intact plant. In the normal, intact, angiospermous plant, a shoot apex in the non-growing, or dormant state, retains the cytological and histological characteristics of the indeterminate meristem, and it may be assumed that such

maintenance is brought about by the biochemical substances furnished it from the subjacent tissues. It may be suggested that the shoot apex, an indeterminate meristem, must be maintained in a particular physiological state that may be indicated visually by its peculiar cytology and histology. This state is indicated functionally by its production of a continuous series of tissues and organs—the open system of growth.

A fundamental question is: can a shoot apex, once it has lost its characteristic cytology and histology after being maintained for a time upon an inadequate culture medium, be caused to revert to the original, functional, meristematic state? It has not been possible to bring about such reversion in the present experiments. However, it must be pointed out that apices were merely transferred to fresh culture medium of the sort that had permitted previously a limited amount of growth. If one assumes that the unfortunate changes in the shoot apices recorded here after they had ceased growth are maturation or differentiation, then the desired return to the meristematic state has a parallel *in vivo*. The process of dedifferentiation, whereby mature parenchyma is known to return to the meristematic state in the production of shoot apices, necessarily occurs in all regeneration. Herein lies another fundamental problem of biochemical differentiation that may be profitably attacked by the methods outlined in this present work.

The tissues subjacent to the shoot apex, which were quickly derived from the lower cut surface that was placed in contact with the agar medium, grew in the manner of a callus. They appeared to be not under control of the shoot apex and did not constitute normal stem tissues during the entire course of the growth. There was always a sharp dividing line between this callus, some of which was continually in contact with the culture medium, and the normal stem that had been later produced by the shoot apex. The factors involved in this peculiar differentiation and manner of growth of this callus are unknown. Certain conjectures, however, can profitably be made. First, the basal tissues of this cultured shoot functioned as absorptive tissues. The absorption and translocation of all materials entering the shoot might have been responsible for these histological peculiarities. Second, these tissues were derived, in part, from the young pith and corpus of the excised shoot apex (cf. inoculum of Fig. 1).

It is conceivable that those tissues had already undergone certain steps in the differentiative process which made them react as they did to the culture medium. It would be advantageous to know whether the edges of the two-layered tunica, which were also at the base of the cut surface, and in contact with the culture medium, reacted in a similar fashion. As pointed out earlier, these basal callus tissues appeared to grow for a time in a manner independent of the shoot apex above them. The presence of the abnormal strands of tissue within the callus, connecting with the normal vascular strands in the stem above it, indicates that the functioning shoot apex may have determined the former at a time after the callus was partly developed. The very interesting results of Camus (1949) may be cited here as a basis for such speculation; he grafted buds into callus cultures that were growing in sterile nutrient medium and found that the buds caused the basipetal differentiation of vascular strands in the callus. Wetmore and Sorokin (1955) did similar research upon the callus culture of *Syringa vulgaris* and reached similar conclusions.

Summary

Excised shoot apices approximately 300μ wide, 300μ thick and 100μ high were grown in sterile culture on medium containing minerals, glucose and various other components. These shoot apices consisted of the dome of meristematic tissues only and had neither foliar primordia nor subjacent maturing stem tissue. Neither natural nor artificial mixtures of amino acids supported growth. Nine amino acids, which naturally occur in the tissues, plus two which do not, added singly in physiological concentrations, supported, with the exception of gamma-amino-butyric acid, no growth. The latter permitted a small amount of growth; this agrees with findings of others who consider it a good nitrogen-source. No effect was noted for indoleacetic acid; 2,4-D was toxic alone, and increasingly so with an amino acid, and stimulated growth of the shoot apex in the manner that resulted in a callus when present with coconut milk. None of the 12 vitamins tested had any effect on growth. Several derivatives of nucleic acid, except for kinetin (no effect), and adenine (toxic) appeared to support moderate growth. Growth of shoots 5–10 cm. long was achieved on medium containing coconut milk and gibberellins. These shoots were found to have produced 7 to 9 foliar primordia, in contrast to the 3–5 in controls. It is concluded that the embryonic

tissue of the shoot apex was stimulated to undergo cell divisions by gibberellins. This amount of growth represented the total capacity of the tissues, for transferring the shoots to fresh medium did not bring about a resumption of it. It is concluded that the tissues of this shoot apex lost their embryonic characteristics by lack of certain essential substances which were not present in sufficient amounts either in the tissues or the medium. Presumably such substances are furnished to the shoot apex by the older parts of the plant. The present results agree with the suggestion that the shoot apex of angiosperms has undergone biochemical differentiation in the direction of loss of synthetic ability. The shoot apex of the angiosperm is thus regarded as advanced, in contrast to the primitive shoot apices of lower vascular plants that retain complete potentiality for biochemical synthesis. Basal tissues of these shoots, parts of which were continually in contact with the medium, grew like a callus. There was always a sharp dividing line between the basal tissue and the histologically normal stem above it. No roots were ever formed from the basal tissue.

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GENETIC DIFFERENCES IN GROWTH RATE AND MATURATION OF RABBITS

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The inheritance of body size in mammals has been a challenge to the geneticist for more than half a century. Specific dwarfing genes are known in a number of species. In general, growth and size appear to be under the influence of numerous genetic factors. On the basis of the multiple factor hypothesis of Nilsson-Ehle, such an interpretation was first made by Castle (2) who referred to it as "blending inheritance." They are now usually interpreted as due to polygenes. Although there are numerous genetic studies of growth and body size within the wide range commonly regarded as normal, the fact remains that size can be described best on a polygenic basis, without clear-cut reference to any of its yet largely unknown specific components.

Early genetic investigations on animals carried out by crosses of large and small races showed that the fundamental difference between them is explainable by a disparity in rate and duration of growth (Castle, 3). Size and age at maturity were considered by Punnett and Bailey (11) to be to some extent transmitted independently.

Studies of human growth have been for the most part non-genetic and primarily designed to isolate clinical factors of growth. They have been largely longitudinal in nature (Tanner, 15) and have shown that there is much variability in time and rate of maturation with little evidence to suggest a relationship between rate of maturing and ultimate body size although shape, especially muscularity, does show some relationship with rate of maturation (Tanner, 16).

Longitudinal data are much more easily secured for animal studies, but analyses to define clearly specific genetic or environmental influence still await a suitable technique. Since growth is a relatively continuous process, it is better characterized by rate of gain than by actual weight. To date this approach has been used relatively little in

animal studies. Weight is a three dimensional measure and generalizations about growth processes from weight curves alone are hazardous and may be misleading (Tanner, 16). It has been pointed out, however, that weight is fairly well correlated with skeletal age (Bayley, 1) such that it may reflect some of the physiological processes within the growing animal. Waters (19) has shown that within breeds of chickens there is some indication of differences in sexual maturation with ultimate body weight depending upon both rate and duration of growth. Ultimate body weight, he found, is not affected by time of reaching sexual maturity. There is thus evidence from both animal and human studies for at least two components of ultimate body size: (a) rate, and (b) duration of growth. Racial or breed differences in specificity of these components in time and in relation to each other have been shown only in the studies by Waters (19).

The present investigation concerns a longitudinal analysis of body weight within three relatively homogeneous populations of rabbits. It was designed to differentiate possible differences in rate of growth and time of maturity and to determine the nature of their interaction in determining ultimate body size. The distribution of maturity types within populations is such as to suggest a possible explanation of the peculiar male-female size relationship of the rabbit which is contrary to that found in most mammals.

In order to avoid possible confusion in terminology, growth will be used only with reference to increase in size. Development and maturation will be used with reference to certain characteristics which are universally achieved by all individuals, the time of appearance varying between individuals, races, and sexes. Thus, maturity, as used here, does not refer to sexual maturity per se, but rather to the ultimate point in time at which velocity of growth approaches zero. Although the two may be related, such relation cannot be demonstrated here with certainty. Weight is used as a measure of body size since differences in body type and conformation are not demonstrable within races, although they are present between races. Its changes reflect the progress of developmental processes within the animal.

Materials and Methods

Since 1951 various races of rabbits have been weighed routinely at birth and every 5 days to 15 days, then 21 days and 30 days, then every 10 days to 60 days, and monthly thereafter to maturity. Pre-

liminary examination showed that most rabbits attain mature size by 210 days. Consequently, only rabbits which survived at least 210 days, and which were not bred or otherwise utilized prior to this age, were used for this study.

The present sample consists of 110 males and 188 females of race III, 73 males and 126 females of race IIIc, a subline of III of New Zealand White origin, and 94 males and 180 females of race X, originating from the small race of Dr. W. E. Castle. This group is composed of 57 males and 140 females which were normal (*dwdw*) and 37 males and 40 females heterozygous for the dwarf gene (*Dwdw*). The inequality of numbers between sexes is due to the impracticability of raising surplus males to maturity. Brother-sister matings have been made where possible, but it has been necessary to resort several times to parent-offspring or first cousin matings to maintain the reproductive capacity of the races. The variations described are indicative of persistent heterogeneity.

The data collected are "pure longitudinal" (Tanner, 15), each animal being weighed at each sampling interval. They are treated without reference to cross-sectional analysis. The method used is the split-plot design (Cochran and Cox, 5) or type I design (Lindquist, 10) which provides an analysis of variance covering in one operation the entire growth span from birth to maturity. It is thus an analysis of the growth curves per se rather than an analysis at any one point in time. The interaction factor between time and any other factor, for example, sex, provides an estimate of the degree to which the shapes of the curves deviate from one another. Valid use of the technique depends upon equal numbers at each sampling interval within groups, but the numbers in different groups need not be the same. Thus only those animals which have been weighed at each interval have been used. The data have been plotted in two ways, linearly—mean weight at successive ages—and as mean rate of gain per unit time—or velocity. The latter is often more informative, especially as it more often reflects physiological events taking place within the animals (Tanner, 16).

Observations

Races III and IIIc

(a) *Linear growth.* Preliminary examination of the data as a whole revealed that, as has been found by others, females are significantly

larger than males ($p = .05$), at least when mature. The difference becomes manifest after 90 days in race III and approximately a month later in race IIIc (Fig. 1). Both races appear to be essentially similar in weight at birth but once the differences begin to appear, they become progressively divergent, IIIc becoming the larger race.

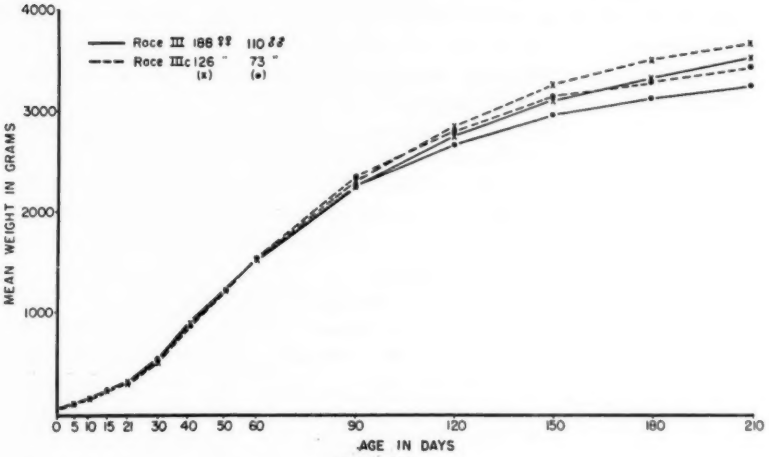

FIGURE 1

Linear growth of races III and IIIc, both males and females. Note that in both races mature females are heavier than males.

Before attempting to study these racial differences in detail, it was deemed necessary to determine whether significant differences were to be found *within* the present races in some measure comparable to those reported for human populations. To do this, a random sample of individual curves of race III was plotted as shown in Fig. 2. Logarithms of weights were not used as they tend to minimize differences whereas our desire was to expose them. Although in general the distribution appears continuous, the curves seem to fall into two groups most widely divergent at 90 days and into four groups similarly separated at 210 days. To test the validity of these groups the entire population, both for race III and IIIc, was typed on the basis of their weights at these two periods.

Examination of the mean curves from the re-sorted data (Fig. 3)

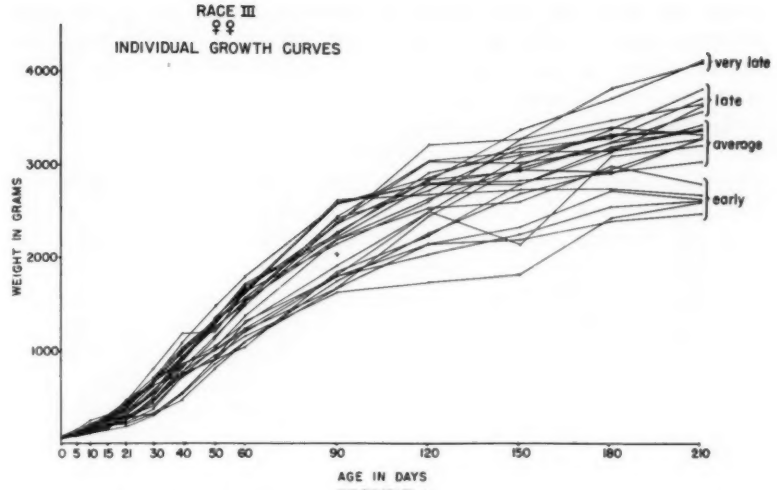

FIGURE 2

Composite sample of individual growth curves of race III animals. Note early separation into two groups centered around 90 days and later separation into four groups at 210 days.

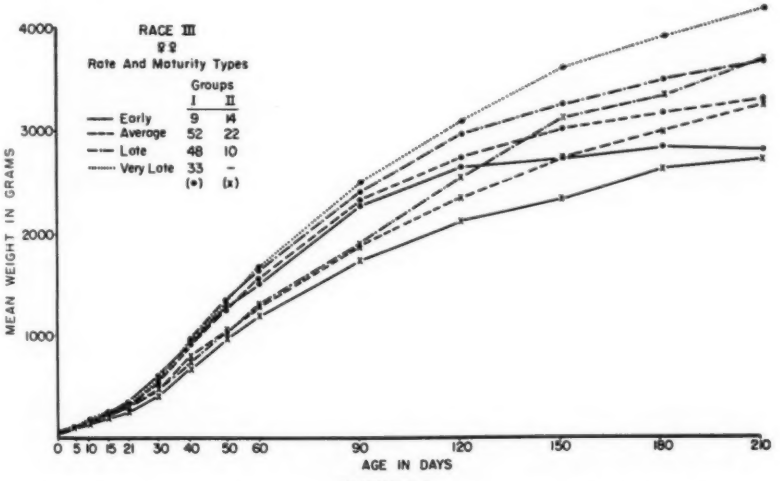

FIGURE 3

Mean weights of race III females of different maturity types and rate groups. Note that maturity groups are the same regardless of whether group I or group II.

shows for each race two groups of animals growing at significantly different rates. These have been designated groups I and II. After 90 days each of these noticeably separates again into four groups which become progressively divergent. These new groups have been called early, average, late, and very late maturers since it appears that the lightest group ceases to grow appreciably after 120 days and the others after 150, 180, and 210 days respectively. The maturity types appear to be the same for both groups I and II. The rate groups and the maturity types are significantly different ($p = <.01$) for both males and females.

Animals of the different maturity types within groups are similar from birth to about 60 days when they begin to diverge (Fig. 3). The greatest divergence occurs from 120 days on. Early maturers slow down markedly, very late maturers continue to grow quite rapidly, and average and late maturers take respective intermediate positions, but with each type tending to show a marked decrease about a month after the preceding type.

Similarly, animals of groups I and II, equal in size at birth, diverge rapidly after 30 days. Within maturity types, group II animals appear to grow somewhat more rapidly after 120 days than do group I animals, so that by 210 days the curves of the same maturity type again converge.

As previously mentioned, the sex difference does not show up until 90 days or later in race III. Prior to this time, males may even be a little heavier than females, but after this their rate of gain falls rapidly. In group I animals the difference begins to appear by 90 days. The slower growing group II animals show no difference until 120 days, a month later than their faster growing sibs. In both groups, the males and females grow at significantly different rates ($p = <.01$).

Analysis of sex deviation within maturity types of race III shows no significant difference except among very late maturers where this difference is significant only at the 5% level. In IIIc the early, average, and late maturers also show only a slightly significant sex difference ($p = .05$).

The distribution of maturity types (Fig. 4) shows that in race III, although average maturers predominate in both sexes, the majority of males are either early or average maturers (78%), whereas females are predominantly average or late (70%). In IIIc there are few early

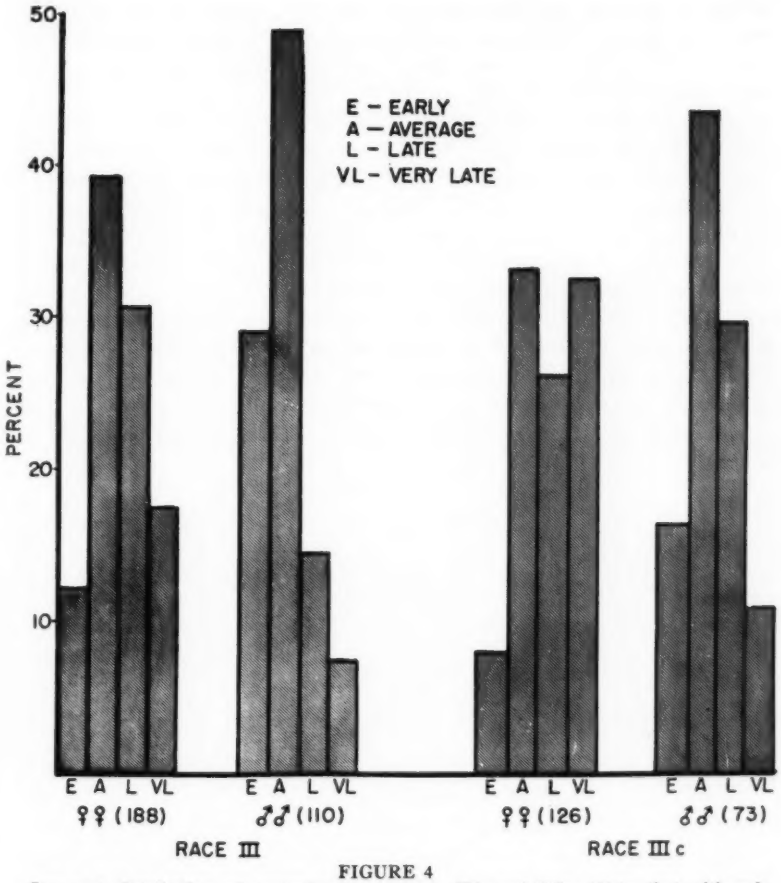

FIGURE 4

Per cent distribution of maturity types, races III and IIIc. Note that although average maturers predominate throughout, there are more later types among females, especially in race IIIc.

maturers. Amongst females of this race there are nearly equal numbers of average and very late maturers with almost as many late maturers. Males are predominantly average maturers (45%), but show also a fairly high percentage of late maturers (29%). Distri-

bution of average and late maturers are thus similar in the two sublines, though there are considerably more late maturing males in IIIc than in III. IIIc has many fewer early maturers and many more very late maturers than III.

(b) *Velocity of growth.* Curves of velocity are shown in Figs. 5 and 6. Fig. 5 shows mean velocity curves for races III and IIIc regardless of rate group or maturity type. Certain characteristics are apparent. First, there is a sharp break in the initial rapid acceleration, sometimes even a decline, between 10 and 21 days. Following this, the rise to peak velocity at 40 days is sharp and the decline thereafter slow at first and then quite abrupt. Between 60 and 90 days there is a break in this rapid decline, after which deceleration is again greater—but not so great as the initial decline—gradually approaching zero. These characteristic changes appear to be consistent as to age of occurrence for both races and sexes, but IIIc has a somewhat greater peak velocity and its rate is sustained somewhat longer.

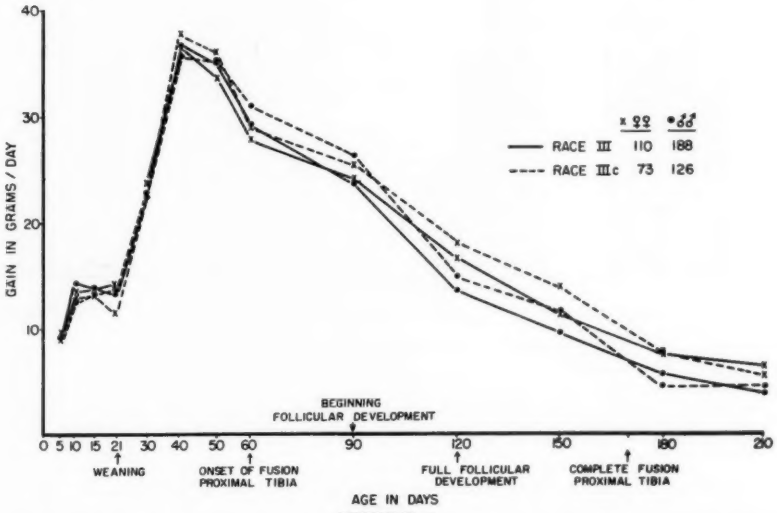

FIGURE 5

Mean velocity of growth races III and IIIc regardless of maturity type. Time of certain physiological and morphological changes is indicated below base line. Note how these are reflected in changes in rate of growth.

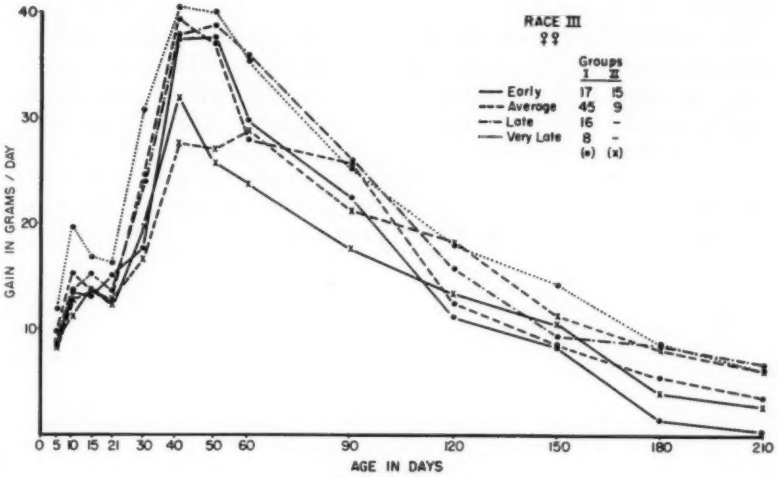

FIGURE 6

Mean velocity of growth within maturity types and rate groups of race III. Note similarity of time of most major characteristics in all curves.

Early rate of growth, particularly up to peak velocity, is very similar for both males and females. Males, however, show a tendency to grow more rapidly than females, particularly during weaning and the initial post-peak decline. From 90 days on deceleration is somewhat greater for males than for females.

Between maturity types (Fig. 6) there is relatively little difference in early velocity, though there is a slight tendency for greater rate of growth with late maturity. Peak velocity is reached for the most part by 40 days. Any increase in rate of growth beyond this age is slight. After the peak, differences in maturity types become more apparent, due to greater and more sustained growth in the later types.

As might be expected, the greatest differences between groups I and II occur between 30 and 90 days, the period of most rapid growth, with group II far below group I. After 120 days there is little difference between the two groups although group II may decelerate somewhat less rapidly. The same sex difference may be seen here as was seen for the population as a whole, the males growing somewhat more rapidly than the females prior to 90 days and appreciably less thereafter.

Race X

(a) *Linear growth.* As race X is a relatively small race the race III standard of classification could not be used and, in fact, it was found that the two genotypes (normal *dwdw,* and heterozygous dwarf *Dwdw*) required different standards. These were set up in the same manner as that for race III.

Analysis of the sexes, regardless of group, again shows that amongst normals (*dwdw*) the males are initially somewhat heavier, but after 120 days fall behind (Fig. 7) due to the significantly different rates

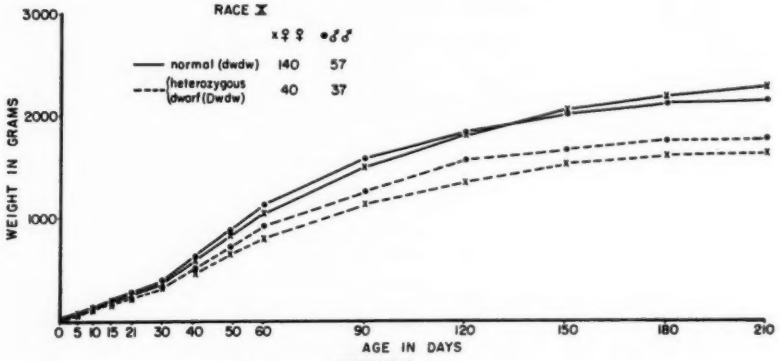

FIGURE 7

Mean weights of race X normals (*dwdw*) and heterozygous dwarfs (*Dwdw*). Note persistent larger size of *Dwdw* males.

of growth for the two sexes ($p = <.01$). Amongst dwarfs (*Dwdw*), however, the males maintain their size supremacy. This difference is significant at the 5% level of probability. Animals of the two genotypes are similar at birth, but become progressively divergent thereafter. The difference between the two groups is highly significant ($p = <.01$).

Instead of two rate groups and four maturity types, race X separates into slow, intermediate, and rapidly growing animals and only early and average maturers ($p = <.01$). Animals within rate groups are similar until 60 days (female *dwdw* 30 days) from which time they become progressively divergent (Fig. 8). Intermediate growers comprise the only group which differentiates into both early and average maturity types. These types are not differentiated in males of

either genotype, however, until 150 days when the early maturers have essentially reached their mature weight, while the average maturers are still continuing to gain. The females, on the other hand, begin to diverge after 90 days. Of the four groups in each genotype, two in the normals are early maturers and two average, whereas in the dwarfs three are early maturers and only one average.

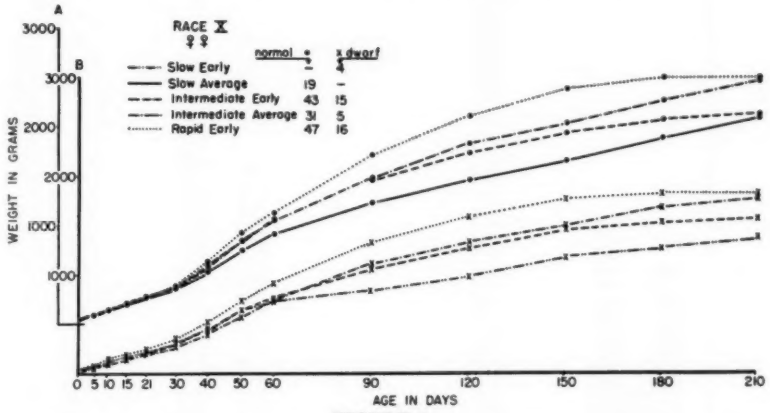

FIGURE 8

Mean weights of race X normals (*dwdw*) (A), and (B) heterozygous dwarfs (*Dwdw*) of different rate and maturity groups. Note similarity of latter third of curves of same maturity types.

Sex differences within types for normals are highly significant for all groups ($p = <.01$). The dwarfs, on the other hand, show no sex difference except for the intermediate early maturers, which are again highly significant.

Distribution of maturity types shows X to be predominantly an early maturing race. This is especially true of the dwarfs (*Dwdw*) with 89% so classified in both sexes (Fig. 9). Amongst normals (*dwdw*) there are more average maturers, about twice as many in males and three times as many in females. In both genotypes, slow growing animals are in the minority, as are the intermediate growing average maturers, with intermediate and rapidly growing early maturers predominating.

(b) *Velocity of growth.* Characteristics of the velocity curves of race X are similar to those of race III (Fig. 10). The early decline

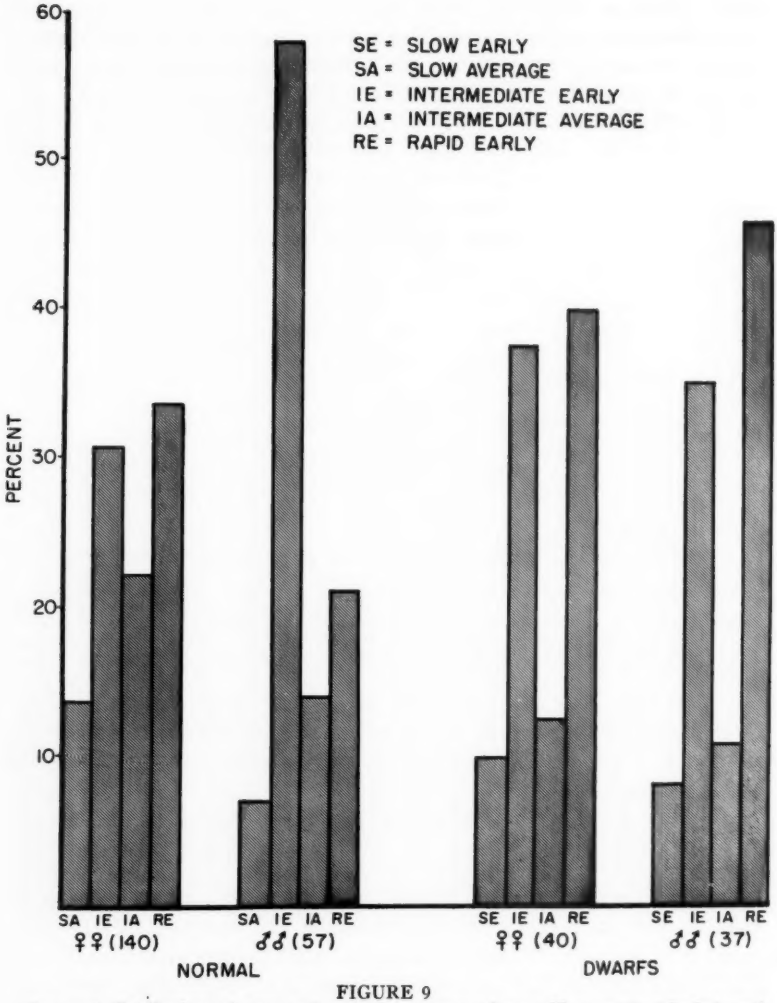

FIGURE 9

Per cent distribution of rate and maturity groups of race X normals (*dwdw*) and heterozygous dwarfs (*Dwdw*). Note that intermediate and rapidly growing early maturers predominate in both genotypes.

due to the decreases in the mothers' milk supply is relatively more pronounced. Recovery is less rapid, but acceleration to peak velocity is very similar for a short distance. Growth subsequently slows again just prior to attaining peak velocity at 50 days. The deceleration

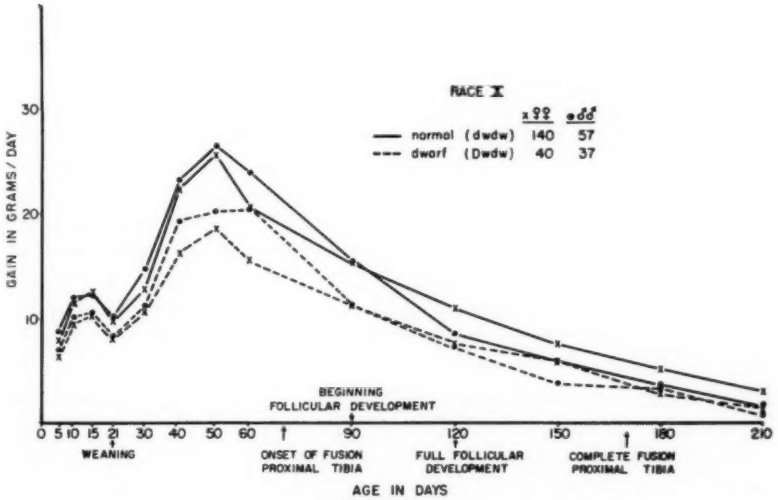

FIGURE 10

Mean velocity of growth of race X normals (*dwdw*) and heterozygous dwarfs (*Dwdw*) regardless of rate group. Compare with Fig. 5.

from peak velocity is more gradual than in III and shows no abrupt changes in its approach to zero. The two genotypes are similar throughout, but with the dwarfs always growing at a slower rate, especially during the period of most rapid growth from 30 to 120 days.

Males of both genotypes appear to grow more rapidly than females, especially in the dwarfs (*Dwdw*), until about 90 days when the growth rate of the two sexes is about equal. Thereafter normal males grow more slowly, while dwarf males continue at about the same rate as the females.

Velocities of growth of the various rate groups, especially in normals, diverge at about 30 days, well before peak velocity (Fig. 11). They continue divergent until 150 days, when all are fairly similar. Thereafter segregation is due to maturity type rather than to rate of growth,

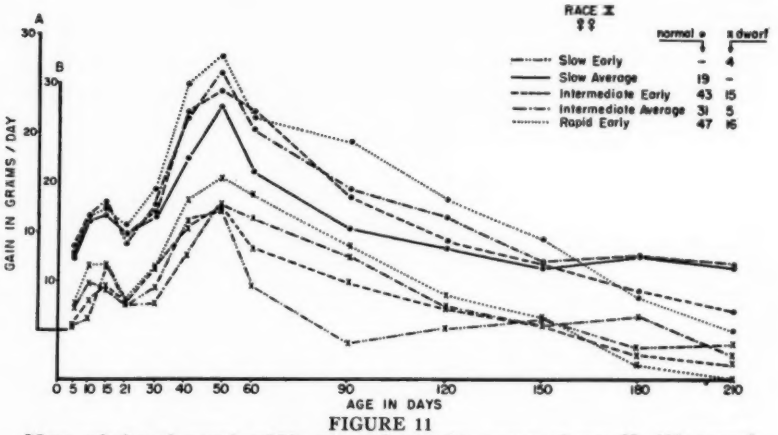

FIGURE 11

Mean velocity of growth within rate and maturity groups of race X, (A) normals (*dwdw*), and (B) heterozygous dwarfs (*Dwdw*). Note close approximation of latter third of curves of same maturity types.

average maturers maintaining somewhat more growth than early ones. The greatest difference in rate curves occurs between 60 and 90 days.

Race X never achieves as great velocity as race III and reaches its peak approximately 10 days later. Once the *decline* in race X normals (*dwdw*) sets in, it is nearly the same as in III up to 90 days, but is relatively less during the subsequent 90 days. Between 180 and 210 days, both races are about the same. The principal effect of the dwarf gene (*Dw*) is that the velocity never reaches the peak of normal rabbits of the race. It declines at nearly the same rate except in males, which approximate the female rate during the last half of the growing period.

Discussion

Application of the technique described in this paper to longitudinal data of the three racial groups, III, IIIc, and X, has demonstrated significant differences in the form of the growth curve. To some extent, these are in harmony with the concept of ultimate body size proposed by Castle, based on his studies in rabbits (3), to the effect that small body size may be associated with slow rate of growth or cell division, whereas large size is associated with a greater growth rate. The data are likewise in harmony with the work of Bayley (1) on human growth where short stature is associated with early maturity

and tall with late. It also emphasizes a fact pointed out by Tanner and Sawin (17) that rapid early velocity "does not necessarily lead to a demonstrable result in the mature animal." In some measure, at least, it supports the idea expressed by Punnett and Bailey (11) that the two characteristics, size and maturity, may be transmitted independently, a conclusion also drawn by Waters (18) in an exhaustive genetic study of growth and sexual maturity in fowl.

Ultimate body size and maturity are, however, end results of developmental processes, some of which may produce their greatest effects very early and which may even be partially or completely obscured by their interaction with subsequent influences and/or with the environment. It is possible factors of this kind that we wish to expose. An example is clearly apparent in a comparison of the velocity curves of races III and X. Early maturing small race X animals appear to mature somewhat later than early maturing large race III animals. The same is true for the average maturers of these two races. Differential patterns of growth of organs and parts have been pointed out in man by Scammon (14) and by Krogman (8). They are probably to be found in other mammals, since localized quantitative differences in growth pattern have been clearly shown in the axial skeleton (Tanner and Sawin, 17; Crary and Sawin, 7; Sawin and Crary, 12), some of which have been found to change with age (Sawin, Crary and Webster, 13). The present data are not such as will define differences in space, as opposed to time, nor will they entirely eliminate the possibility of variation of an exogenous nature. They do, however, clearly indicate the existence of influences acting differentially in time and the relationship of these timed differences to race, sex, and specific genes provides evidence that a substantial part of the variation is genetic.

In mammals there seem to be at least four chronological points at which the growth curve of the species may be expected to vary. The species variations described by various investigators have been brought together by Tanner (16). It is interestng to note the manner in which these points are influenced by these racial, genotype, and sex groupings.

The characteristics of the earliest part of the velocity curve, at birth, are determined by birth trauma and the necessary accommodation of the individual to extra-uterine environment. This our present

data do not cover. The second portion represents the adjustment period between infantile and juvenile modes of food supply and activity, or from maximum dependence on maternal care to complete independence. In our data this finds expression in characteristic velocity depressions at 21 days (Figs. 5, 6, 10, 11). Such declines tend to be greater in the slower growing animals which may also take longer to regain the previous rate of growth. The tendency is apparent in race III (Fig. 6) in which both group II curves are among those showing the greatest effect. In race X (Fig. 10) dwarf males and females show the greatest decline and the decline in X as a whole is greater than in III.

The third and, in our data, most significant variable is peak velocity. Not only is there present in these data the generalized racial difference in growth rate described by other authors but, as shown by Tanner for different species, there is also a racial difference in the time that peak velocity is reached; this is ten days later in X than in III.

The fourth characteristic of the human and primate growth curve, the prominent adolescent spurt, seems to be non-existent in the rabbit, (as found also by Tanner in species other than primates) unless the slight interruption in declining velocity between 60–90 days to be noted in Fig. 5 (races III and IIIc) may be so interpreted. This interruption in decline occurs when growth is still very rapid, at about the time Tanner has postulated that it should occur. In Figs. 5 and 10 we have shown certain of the important developmental landmarks in relation to the velocity curves. Of these, follicular development is the least well established. From preliminary observations it seems to occur in rabbits generally between 90–120 days, after which sexual maturity is established. Puberty, which follows the adolescent spurt in man, is by definition associated with maturity of the gonads. It is also associated with the degree of maturity of the epiphyses. Preliminary investigations of small samples of rabbits have shown that onset of fusion of the proximal epiphysis of the tibia takes place at 60 days in race IIIc and 70 days in race X (Crary, 6). This racial difference is of the same magnitude as that found in connection with peak velocity. Onset of fusion of the proximal epiphysis of the tibia coincides with the beginning of the "adolescent spurt"; beginning follicular development with its end; and full follicular development

occurs a month later. Thus, while there is no highly significant "adolescent spurt" in the rabbit, there are aberrations in the declining velocity curve which in some degree match both chronologically and biologically this characteristic of the human curve.

Although the sample populations used are too small to test adequately this characteristic within the maturity groups, Fig. 6 further shows some indications that the "spurts" tend to occur in all groups; if this is correct those of the late maturing groups occur later than those of the early maturing. It also should be noted in this connection that slower growing group II animals do not show this phenomenon. This is also true in race X, which as a race grows more slowly than III. Whether absence of the "spurt" can be explained on the basis of slow growth or whether it is obscured because the time intervals (in months) at which the data were collected are not adequate for its demonstration in the slower growing race can only be determined by special study. Any evidence of this phenomenon is apparently obliterated where (*dwdw*) and (*Dwdw*) populations are combined.

It is generally accepted that in the rabbit the female when mature is the larger of the two sexes. Castle (4), however, stated that the sex difference in weight for his small race was possibly not significant. Similarly, Punnett and Bailey (11) found little size difference between sexes either in their small Polish race or in their original large Flemish animals. Both after inbreeding and in the F_1 populations, however, the females were much heavier. These authors attributed the difference to heterogeneity of the original stock.

The present data on three races of rabbits show a generalized tendency for adult rabbit females to be the heavier. Significant variations in the growth pattern are noted which, if genetic in origin, could explain this peculiarity in sex weight. The sex difference appears to result from the tendency of the males to grow somewhat more rapidly than females during the early part of the growth cycle, especially in race X, and to decelerate more rapidly in the latter part, but there is some variation in time of appearance of this difference. Since late appearance of sex difference is found in both the largest animals studied (which grow at a relatively rapid and prolonged rate) and also in the slower growing small animals, such delay of sex difference must be due to some inherent racial characteristic over and above the rate of growth or size per se.

Since little difference can be demonstrated between the sexes within any maturity type, the difference found in the population as a whole (which contains some of all the maturity types) must depend upon the distribution of these types. When the sexes are compared on this basis, the males in race III are predominantly early and average maturers whereas the females are average to very late maturers. The further variation in the relationship of the sexes present in the heterozygous dwarfs (*Dwdw*) of race X is of some significance in this connection. Here, as a result of a single gene (*Dw*) affecting body size, the male maintains a significant weight superiority throughout the life span, indicating that this gene has a much greater retardation effect upon growth of females than on that of males. Latimer and Sawin (9) have shown also that there is a differential effect of the *Dw* gene upon most of the internal organs of the two sexes in these animals. Since this gene has been much studied, but without manifesting direct evidence of its association with the sex chromosome, it may be assumed that these growth differences are extra chromosomal and probably associated with the divergent sex endocrine mechanism.

Thus it may be seen that major changes in the growth pattern both within and between these three races seem to revolve around the time of puberty. Prior to this time, males tend to be somewhat heavier, whereas thereafter females are the larger. Similarly, differences in rate of growth are most pronounced before puberty, those in time of maturity thereafter. These two factors, therefore, are independent of one another though they act cumulatively in determining the ultimate size of the animal. To some extent, they act differentially upon the two sexes, but it is probable that some other factor or factors are responsible for the ultimate sex difference.

The methods of analysis and results described above have revealed the existence of growth and maturity values specifically localized in time, whose relationships to race, sex, and specific gene strongly suggest a genetic origin. The specificity of gene action, however, remains to be demonstrated by standard genetic procedures of hybridization and subsequent test matings accompanied by similar statistical procedures. The major value of these studies lies in the evidence of the usefulness of the techniques and of their application to the animal material now under similar investigation.

Acknowledgment

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Summary and Conclusions

A longitudinal analysis of body weight involving 497 rabbits of race III (including two sublines) and 274 of race X (including two specific genotypes, normal (*dwdw*) and dwarfs (*Dwdw*)) demonstrates characteristic timed variations in growth rate and maturity peculiar to race, sex, and specific genotype, by inference indicating their genetic origins. Race III is found to be slightly more advanced at all ages than its subline IIIc and significantly more so than race X. Significant differences in velocity of growth prior to puberty at 120 days and in time of maturity thereafter permit classification of all individuals into two groups with respect to prepubertal rate and four groups with respect to age at maturity indicative of independence of these characters. Since little sex difference can be demonstrated within maturity types, such differences or their lack are attributed to the distribution of the types within the populations as a whole. This may explain conflicting reports on the sex differential of the rabbit. The single gene *Dw* is found to induce a sex differential in body weight. Curves of velocity show that there are different racial characteristics of growth affecting time of peak velocity, X being later than III. They also show an arrest in the decline of velocity prior to puberty, especially in faster growing animals, which may correspond to the "adolescent spurt" of primates. It appears to occur later in the late maturity types. The racial differences, together with distribution of maturity types and rates of growth within populations, may account for inconsistencies noted in observations of others.

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X-RAY EFFECTS ON EMBRYOS OF THE PARADISE FISH, WITH NOTES ON NORMAL STAGES*

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INTRODUCTION

This paper is the report of a histological and morphological study concerning the age-sensitivity relationship as seen in the embryonic organs of the Paradise Fish, *Macropodus opercularis* (Linnaeus). Many studies of the critical periods of sensitivity during embryological development in the several vertebrate classes have been published (O'Brien, 1956). However, the effects of deleterious physical and chemical agents upon specific organs throughout all stages of development have not been described in a manner as detailed as would seem desirable. Except for Russell's papers (*e.g.*, 1956) on the mouse, few of them have been as detailed as was attempted in this study. Solberg (1938), Welander (1954), and Rugh & Clugston (1955) studied the effects of radiation upon the development of the fishes *Fundulus* and Salmon, and provided a good foundation for the present study, which was to accurately note the periods of time when specific abnormalities might be induced in various organs and tissues. Much information is available on the normal organogenesis of certain teleosts (*e.g.*, Wilson, 1889; Oppenheimer, 1937; Battle, 1944; Tavolga, 1949, 1950; Hodges & Behre, 1953; Mahon & Hoar, 1956; and Hisoaka & Battle, 1958.)

In this study three objectives were accomplished: (a) Microscopic observations of the normal development of twelve selected organ systems; (b) Study of the effects of X-irradiation upon those organ systems, noting the critical periods during which certain abnormalities were consistently produced; and (c) Observation of some interesting features of development in which *Macropodus* differs from the typical teleost patterns.

* Work performed under contract No. AT(11-1)-349 for the United States Atomic Energy Commission.

Materials and Methods

Paradise Fish (Family Anabantidae) are easily reared in the laboratory. The adults withstand a temperature range from about 55 to 90 degrees Fahrenheit and are fairly resistant to minor changes in pH and salt concentration. Their supplementary air-breathing apparatus makes them somewhat independent of oxygen tension. A well-tended breeding pair will produce from 500 to 1200 offspring per mating and will mate from one to three times each year. Fertilized eggs float to the surface of the water and are easily collected. The eggs develop quickly and hatch in 1½ days. Their vitelline membranes are sufficiently transparent to permit some observations of their development.

Approximately 20,000 embryos at various developmental stages from fertilization to hatching were exposed to X-radiation (Fig. 1). The dose of 1000 roentgen units used in most of this experiment was effective enough to induce decisive changes in developmental patterns, kill a moderate number of the embryos, and leave some survivors for further study. Heavier doses of 2500 to 5000 r given principally at age 19 hours were used for mortality and tissue studies. Specimens were irradiated while floating at the surface of water in a plastic thimble one centimeter in diameter. Irradiation was performed at 434, 215 or 80 r/min (85 KVP, 5 MA).

More than 1500 specimens were fixed in Bouin's solution at varying times after irradiation, 1000 of which were sectioned at 4-6 microns and stained with hematoxylin and eosin or with Wright's stain (see Fig. 1). The use of cedarwood oil as a clearing agent helped preserve the friable yolk sac with little crumbling. Other specimens were preserved *in toto* in 70% alcohol or in balsam.

Summary of Normal Development (27° C.)

In this outline some of the principal developmental features are listed at the time of their first appearance in the embryo. In the ensuing sections twelve organ systems are presented in greater detail.

Age	Feature
	Unfertilized egg, about 0.7 mm diameter. Cytoplasm evenly dispersed throughout yolk. Large oil vesicle (about 1/3 of the egg volume) located largely in vegetal hemisphere.

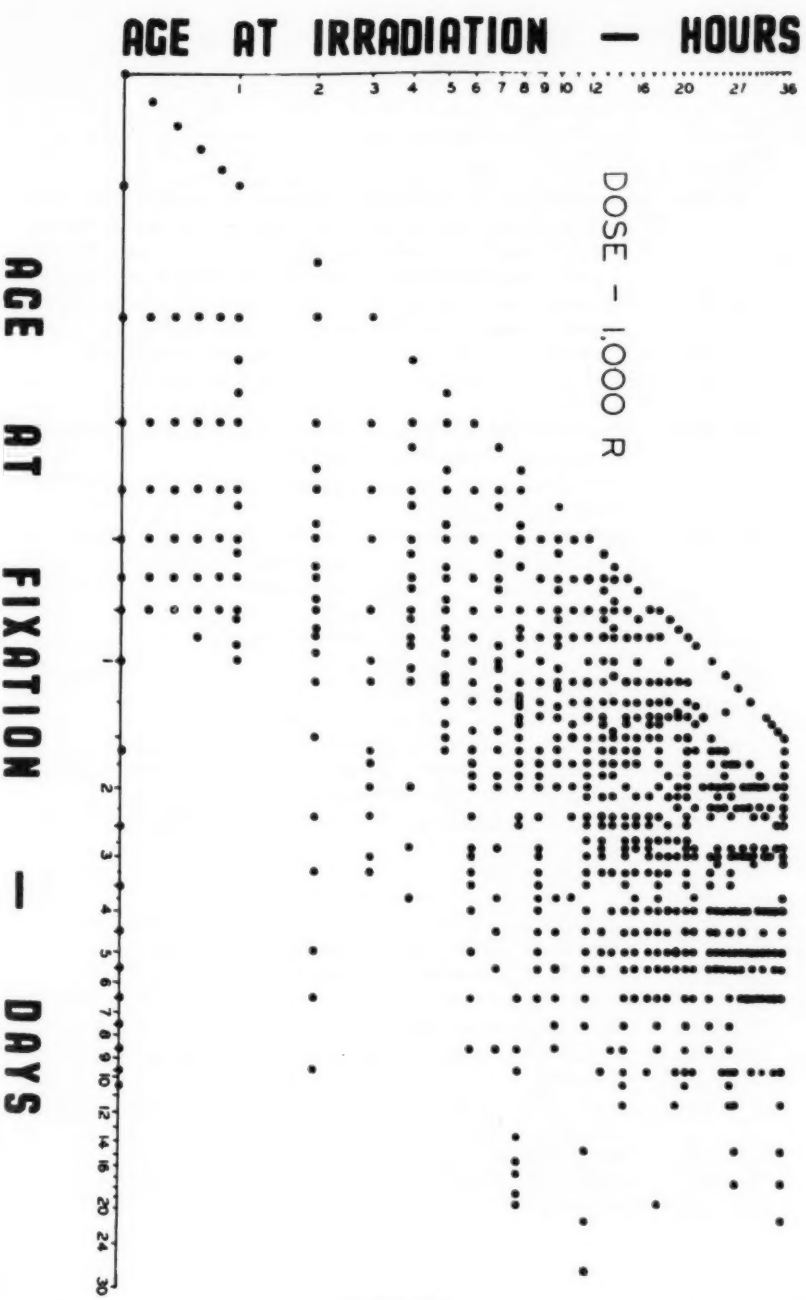


FIGURE 1

Specimen record. Each dot represents one or more specimens irradiated with 1000 r at some stage between fertilization and hatching and subsequently fixed.

AGE	FEATURE
0 Hrs.	Fertilization. Cytoplasm condenses at animal pole forming a high disc. First cleavage occurs after about 20 minutes and next few follow at same interval. Small amount of yolk material included within cells at each cleavage.
3 Hrs.	Blastula stage. Blastomeres begin migration over surface toward vegetal pole. Periblast cells differentiate.
6 Hrs.	Blastodisc elongates. Periblast migrates peripherad.
7 Hrs.	Gastrulation begins. Kupffer's vesicle appears. Neural keel formed.
8 Hrs.	Hemocytoblasts begin to differentiate from mesenchyme. Germ layers formed. One somite.
9 Hrs.	Notochord formed at caudal end.
10 Hrs.	Three to four somites.
11 Hrs.	Forebrain distinct. Optic buds. Dorsal neural furrow. Cranial flexure. Six to seven somites.
12 Hrs.	Otic placodes. Eight to nine somites.
13 Hrs.	Optocoel. Neural furrow closes.
14 Hrs.	Prosencephalon and mesencephalon cavitate.
16 Hrs.	Otic vesicle cavitates. Optocoel invaginates. Lens placode. Twelve to sixteen somites.
17 Hrs.	Wolffian ducts. Pigment cells in trunk.
18 Hrs.	Optic lobes of mesencephalon. Pronephric capsule.
19 Hrs.	Lateral line anlagen. Pigmented otocyst. Infundibulum.
20 Hrs.	Tail free from yolk. Olfactory placodes.
21 Hrs.	Posterior thickening of gut endoderm. Kupffer's vesicle disappears.
22 Hrs.	Erythroblasts.
23 Hrs.	Five brain regions evident. Heart tube.
25 Hrs.	Glomerulus. Blood vessel walls form. Notochord cells vacuolate. Muscle fibers differentiate. Epiphysis.
26 Hrs.	Pharynx and esophagus endoderm condense.
28 Hrs.	Wolffian duct joins cloaca.
29 Hrs.	Pigmented retina. Heart tube bends.
30 Hrs.	Otic ganglia. Hematopoiesis in gut submucosa and dorsal mesentery.
32 Hrs.	Germ cells distinct. Mid and hind gut cavitate. 5, 7, 9, 10 Cranial ganglia.
33 Hrs.	Endolymphatic duct. "Semilunar" valves.
35 Hrs.	Erythrocytes acquire hemoglobin. Pigmented sclera.
36 Hrs.	Hatching. Pharynx cavitates. Anus opens.

AGE	FEATURE
37 Hrs.	Retina 4-layered.
39 Hrs.	Otic maculae and semicircular canals.
40 Hrs.	Atrio-ventricular valves.
41 Hrs.	Liver diverticulum. Optic chiasma.
44 Hrs.	Gill arches.
46 Hrs.	Anal plug removed.
48 Hrs.	Esophagus cavitates.
51 Hrs.	Otocyst partitions.
54 Hrs.	Heart begins S-bend.
60 Hrs.	Hematopoiesis in liver and spleen. Pigmented iris. Germ cells migrate ventro-anteriad.
69 Hrs.	Stomach distends.
90 Hrs.	Mouth opens.
100 Hrs.	Hematopoiesis in pronephros. Gut coiling starts. Yolk largely absorbed.
1 Mo.	First mesonephric tubule.

EFFECTS OF IRRADIATION UPON SPECIFIC ORGANS AND SYSTEMS

Twelve organs, systems, or tissues are presented in the approximate order of their decreasing sensitivity to irradiation. They are:

(1) Blood and Hematopoietic Organs
(2) Eye
(3) Central Nervous System
(4) Germ Cells
(5) Muscles
(6) Gut
(7) Heart
(8) Ear and Lateral Line
(9) Pronephros
(10) Olfactory Organ
(11) Notochord
(12) Pigment Cells

The results are presented graphically and verbally. In the left column of each graph a few landmarks of development are listed; in the right column are bracketed periods of time when specific anomalies may be produced by exposure to irradiation. Exposure to 1000 r units of X radiation during these periods produces the results indicated. Although the anomalous patterns described are characteristic, some specimens do not display the typical damage patterns. The severity of each anomaly is variable and the frequency of induction ranges from 10% to 90%, with an average of about 50%. That is, about one-half of the embryos exposed to 1000 r develop the specific anomalies under consideration. Abnormalities are noted in almost every

organ and tissue, but are not recorded in this report unless they are consistently produced in at least 10% of the exposed specimens.

The general results of irradiation with 1000 r are to delay any or all features of development. This is not always indicated in the following pages but should be kept in mind in addition to the described phenomena. The delay in development seems to be due to a suppression of mitotic activity, with a subsequent inhibition of differentiation (cf. Figs. 6 and 7, 17 and 18). Shortly following exposure the mitotic cells become damaged (Figs. 8-11). At the 1000 r dose the necrotic cells are usually sloughed off into the nearest cavity, (Figs. 13, 14) after which apparently normal mitotic activity and some further differentiation ensues (Figs. 19, 20). In most cases differentiation occurs while the organs are smaller (*i.e.*, contain fewer cells) than normal. The resultant organ is thus smaller and younger-looking than the control [note small neural tube (Fig. 5), straight gut in Fig. 31], but otherwise apparently normal in morphology and function. The small size sometimes affects neighboring organs. For instance, the dorsal surface of the notochord sheath may buckle when adjacent to a shrunken neural tube.

A heavy dose of 5000 r usually produces necrosis, followed by dedifferentiation (Figs. 9, 14, 23, 25).

A dose of 1000 r given during the first six hours of development is sufficient, in most cases, to stop development at the high blastodisc stage, prior to gastrulation. Only about one percent survive to hatching, but a few live as long as a week (Fig. 16). Exposure during early gastrulation (7-9 hours) usually stops development in the "keel" stage, prior to neurulation. After this, 1000 r usually is not sufficient to stop total development, but occasionally kills the organisms as a result of general necrosis resulting from damage to individual sensitive organs (Fig. 2-5).

Blood and Hematopoietic Organs

Normal Developments

One of the earliest of all cell types to differentiate is the hemocytoblast which drops into the subgerminal cavity between the blastodisc and yolk mass at eight hours. This hematopoietic activity is continued by the undifferentiated body mesenchyme until hatching. Hemocytoblasts become randomly distributed around the periphery of the

egg between the yolk and epidermis. They continue cyto-differentiation until, at 22 hours, they may be classed as erythroblasts. At this time they are found in a narrow band along the ventral surface of the anterior body axis in intimate contact with the yolk, where they differentiate as the heart tube. The outer cells flatten to become the vessel walls. The circulating cells retain mitotic activity until differentiation into erythrocytes begins at 34 hours. The erythroblasts become elongated, agranular and pink with the tinge of hemoglobin. They become fully mature about 3 days later. At hatching time (36 hours) blood cell formation has commenced in the walls of the sinus venosus, in the submucosa of the gut at the site of the presumptive liver, and in the dorsal mesentery where the spleen will arise. Hematopoiesis in the mesenchyme ceases at about this time. The heart retains its hematopoietic activity only through the first day after hatching, but the spleen and liver continue active through the larval period. The mass of hematopoietic tissue surrounding the pronephros begins to differentiate erythrocytes on the third day.

Radiation Results

Of all embryonic tissues the hematopoietic tissues are the most sensitive to irradiation. Exposure during the first seven hours destroys almost all blood cells (Fig. 34). The only cells left in circulation by the third day are a few immatures that have subsequently differentiated from surviving hematopoietic tissue, and these cells tend to not fully differentiate into erythrocytes. Exposure between the seventh and sixteenth hours of development reduces the number of RBC that subsequently appear; the earlier the exposure the greater the reduction. These blood cells disappear from circulation in seven to eight days, after which few or none may be found in circulation. Two explanations probably account for this. First, the RBC appear to have a short life span of seven to eight days. Second, whereas all hematopoietic tissue is destroyed by 1000 r during the period of gastrulation, the erythroblasts that have already commenced differentiation are fairly resistant and survive for a week. Thus, embryos exposed at this time live only for a maximum period of about two weeks and probably die because of a lack of circulating cells. The need for oxygen-carrying cells apparently is not critical during the first week of development.

Hours of Development	*Normal Development*	*Radiation Results*
0—		...Very few surviving blood cells; these do not differentiate. (Fig. 34)
5—		
10—	—Mesenchymous hematoblasts delaminate into subgerminal cavity.	...Fewer RBC than control.
15—		
20—	—(Fig. 33)	...Rapid necrosis of circulating RBC.
	—Erythroblasts.	
25—	—Blood vessel walls form.	...Reduction up to 50% in number of maturing RBC.
30—		
35—	—RBC cytodifferentiation; hemoglobin.	
40—	—RBC formation in sinus venosus wall, gut submucosa and presumptive spleen and liver.	
45—		
50—		
55—		
60—	—Hematopoiesis ceases in submucosa and sinus venosus, increases in liver and spleen.	
65—		
70—		
75—		
80—		
85—		
90—		
95—		
100—	—Hematopoiesis commences in pronephros.	

The hematopoietic tissues of the sinus venosus, gut submucosa, liver, spleen, and pronephros are selectively sensitive during their periods of mitotic activity.

The circulating cells display two critical periods of specific sensivity. The first of these is at 17-19 hours, just prior to the stage when the hematocytoblasts may be recognized as erythroblasts. Exposure at this time results in rapid necrosis within a few hours. The other period is between 23 and 35 hours, prior to the transition from erythroblast to erythrocyte. Exposure at this time reduces the number of RBC that mature. Inasmuch as neither of these periods is characterized by mitotic activity, these sensitivities are presumably related to differentiation rather than mitosis.

Eye

Normal Development

The optic bud first projects laterad from the forebrain at 11 hours and soon curves cuadad. The mesenchymal mass condenses and develops an internal cavity, the optocoel. This invaginates at 16 hours, at which time the lens placode differentiates from the nervous layer of ectoderm. Two hours later the lens begins migration and, by 22 hours, is completely enclosed within the optic cup. The lens and retina commence histodifferentiation at 33 hours. Pigment appears in the retina at 29 hours, the sclera at 35 hours, and in the iris at 60 hours. Eye movement begins the third day after hatching.

Radiation Results

The eye ranks with the forebrain as one of the more radiosensitive parts of the body. Irradiation with 1000 r at almost any stage from fertilization to hatching results in anomalous growth of the eye (Figs. 16, 31). Cellular pycnosis appears within 12 hours after exposure (Fig. 18), followed by necrosis and loss of cells into the optic cavity by 24 hours. Mitosis, if it is resumed, is very active, resulting in the restoration of a fairly normal eye after 48 hours (Figs. 20, 21). There are five specific types of damage which have sensitive periods. (1) Exposure between 10-16 hours suppresses the evagination of the optic bud (Figs. 3, 31). (2) During this same time the optic cup, when formed, is caused to develop with walls of uneven thickness. (3) Exposure between 14-24 hours and again after 32 hours results in a

EYE

Hours of Development	*Normal Development*	*Radiation Results*
0—		
		Lag in all phases of development. (Fig. 16)
5—		
10—	—Optic buds project laterad.	
	—Optic buds grow caudad.	
	—Optocoel formed.	
		Optic cup suppressed, uneven. (Figs. 3, 31)
15—	—Lens placode; vesicle invaginates.	
		Clumping after 72 hours.
	—Lens begins migration into opt. cup.	
20—		
	—Lens enclosed within optic cavity.	
	—Lens detach from ectoderm; optic stalk hollow, complete.	
25—	—Lens capsule differentiation.	Breaks in retina. (Figs. 20, 21).
	—Optic stalk solid.	
30—	—Pigment in retina.	
		Inner nuclear layer sensitive. (Fig. 18)
	—Retina and lens histodifferentiation.	
35—	—Sclera pigmented. (Fig. 24)	Clumping after 72 hours.
	—Retina four layers thick.	
40—	—Optic chiasma forms.	
45—	—Retractor lentis.	
50—		
55—		
60—	—Iris pigmented.	
65—		
70—		
75—		
80—		
85—		
90—		
95—		
100—		

clumping of retinal cells. This clumping appears after about three days and persists less than 24 hours, followed by recovery. (4) Exposure at any time after 17 hours results in breaks in the rod and cone layer of the retina which, however, do not appear until after about three days (Figs. 20, 21). The reasons for the relatively long latent period prior to the appearance of this and the previous symptom are unknown. (5) The inner nuclear layer of the retina is specifically sensitive to irradiation between 25 hours and hatching, rapidly becoming necrotic (Fig. 18).

Exposure to 5000 r at 19 hours promptly stops mitosis (Fig. 9), and is followed by 10-12 hours of cellular necrosis and sloughing of cells into the optic cavity (Figs. 10, 11). After this the eye continues to degenerate by cellular migration and rearrangement, with little continuation of differentiation (Figs. 23, 25). The lens and pigment continue almost normal differentiation.

No period has been found when the lens is sensitive to 1000 r.

Central Nervous System

Normal Development

The central nervous system is the first definitive organ system to appear in the developing embryo. At the time of gastrulation at seven hours the keel first appears as a longitudinal streak, 5-8 cells deep. It is first organized at the posterior end and within two hours extends to the anterior end. At 11 hours the prosencephalon and optic buds become distinct and the dorsal neural furrow is formed in the cord region. The furrow remains open for two hours and does not contribute directly to the neurocoele. At 13 hours the optocoel first forms, followed shortly by the prosocoele. These and the other neural cavities open by cavitation *in situ* rather than directly from the neural groove. These cavities open broadly for a time and then, prior to hatching, begin to close, becoming dorso-ventral slits. The three primary brain regions are visible by 19 hours, and all five at 23 hours. The brain commences to flex around the yolk sac at 20 hours, and does not straighten out until the third day after hatching when the head lifts up from the yolk (Fig. 2).

Radiation Results

This system is highly sensitive at all stages of development. Devel-

CENTRAL NERVOUS SYSTEM

Hours of Development	*Normal Development*	*Radiation Results*
0—		
		..... Keel suppression.
		..... Gross contortions. (Figs. 16, 29)
5—		
		..... Posterior lag.
	—Gastrulation and keel formation.	..... Infundibulum suppression.
	—Neural tube length of embryo.	
10—	—Optic buds, prosencephalon, dorsal neural furrow.	..... Optic cup differentiation suppressed. (Fig 3)
	—Optocoel, neural furrow closes.	
	—Prosencephalon cavity.	..... Optic lobe suppression. (Fig. 12)
15—		
	—Optic lobe; thin-roofed myelenceph.	
	—3 brain regions visible externally.	
20—	—Infundibulum; flexion; 3rd vent.	
	—Op. lobe, myelenceph, and cord cavitate.	
	—5 brain regions visible externally.	
25—	—Epiphysis; optic lobe cavity wide.	
	—Op. lobe cavity begins to narrow; white matter ventral brain and cord.	..... Lag in neurocoele closure.
30—	—Myelocoele and cord cavity narrow.	
	—Cranial ganglia 5, 7, 9, 10.	
35—	—White matter in lateral telenceph. and diencephalon. (Fig. 24)	
40—		
45—	—(Fig. 13)	
50—		
55—		
60—		
65—		
70—		
75—		
80—		
85—		
90—		
95—		
100—		

opment of the keel is suppressed by irradiation at any time prior to its formation at seven hours. The posterior end of the neural tube is very sensitive from 4-9 hours, as evidenced by cellular necrosis. Gross contortions of various types (Figs. 16, 29), such as lateral displacement of the dorsal half of the brain, are produced by exposure at 4-6 hours. The development of the infundibulum may be suppressed between 7-9 hours, and the eye may be suppressed during 10-16 hours (Fig. 3). The optic lobes of the brain, from 10 to 19 hours, are more sensitive than any other regions of the nervous system at any time (Fig. 12). Exposure during this period produces necrosis within a few hours, followed by sloughing of cells into the neurocoele. Irradiation between 24 and 33 hours delays the narrowing of the brain cavities for several hours.

Germ Cells

Normal Development

Shortly before hatching, at 32 hours after fertilization, the germ cells become distinguishable and are characterized by their relative clearness and their large, round nucleoli They appear as a small cluster of about two dozen cells located between the pronephric ducts and the gut, close to the cloaca. Two days later they begin to migrate forward along the dorsal mesentery. By the third day they are scattered singly or in small clusters along the mesentery from the swim bladder to the posterior extremity of the abdominal cavity. At the rear they lie just ventral to the pigmented layer of the peritoneum at the root of the mesentery. As they move forward they drift ventrad along the lateral surfaces of the mesentery.

Radiation Results

The germ cells apparently undergo an initial period of proliferation prior to the completion of gastrulation at 17 hours, during which time they are susceptible to radiation damage. Exposure during this early proliferation period significantly reduces the number of germ cells that subsequently appear. The earlier the stage when treated, the fewer the surviving cells. Those which are exposed at 17 hours or later show little or no decrease. As much as 5000 r at 19 hours produces no demonstrable effect.

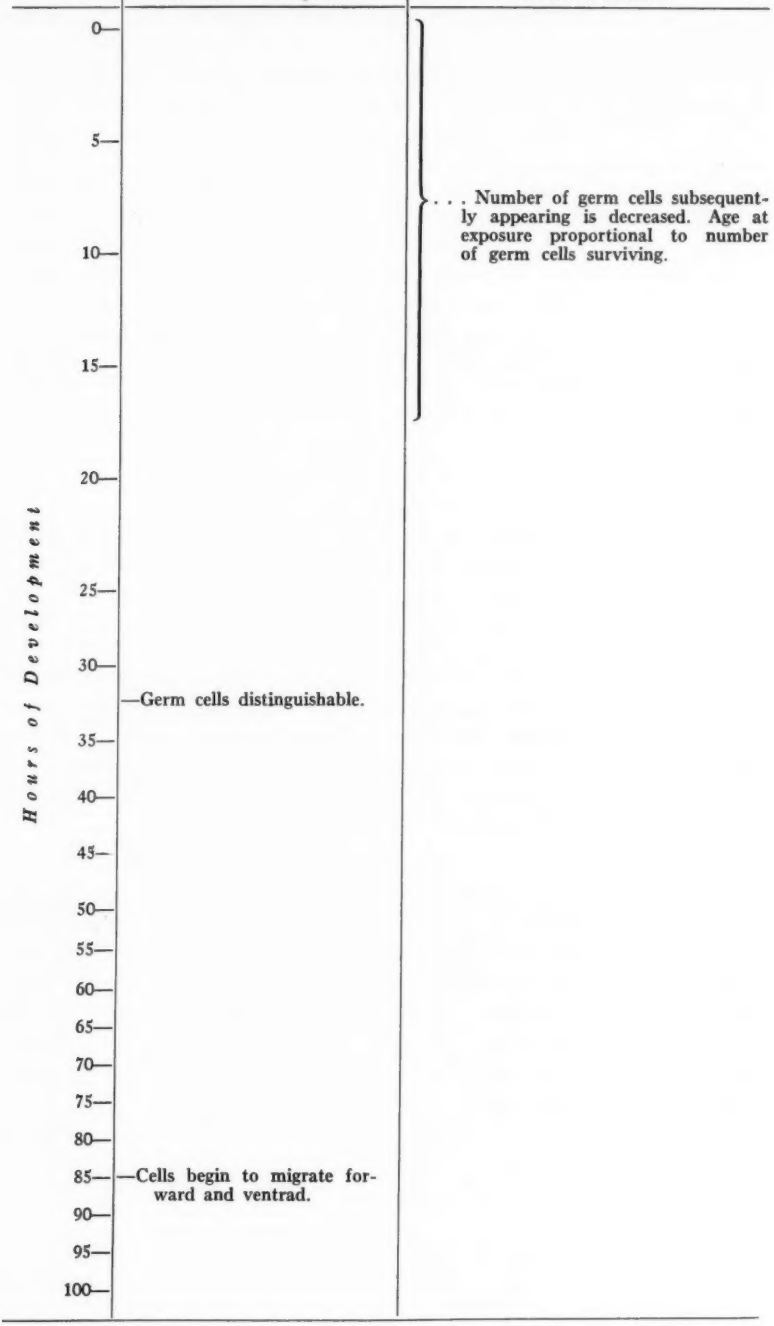

Normal Development
Radiation Results
Hours of Development
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
—Germ cells distinguishable.
—Cells begin to migrate forward and ventrad.
. . . Number of germ cells subsequently appearing is decreased. Age at exposure proportional to number of germ cells surviving.

TABLE I
NUMBER OF GERM CELLS PRESENT IN PRIMORDIAL GONADS

Age at irradiation	48 hours after irradiation	72 hours after irradiation	96 hours after irradiation
Control:	23	18	20
3 hours:	—	—	4
5 hours:	1	3	—
7 hours:	4	—	1
9 hours:	10	14	8
11 hours:	9	15	11
13 hours:	—	9	—
15 hours:	—	4	9
17 hours:	20	16	19
19 hours:	18	21	24
21-37 hours:		Approximately normal	

MUSCLES

Normal Development

The first somites are visible at eight hours, soon after commencement of gastrulation. There is some ensuing rearrangement of cells, but the differentiation into muscle fibers is not distinct until 25 hours. Striations first appear at 34 hours, although muscle movement occurs 3-4 hours prior to this.

Radiation Results

The muscle tissue is susceptible to damage at all stages between fertilization and hatching, the period of greatest sensitivity being prior to the formation of the first somite at 8 hours. Following exposure at any age there is some pycnosis, largely at the edges of the developing myotomes, followed by dedifferentiation of the muscle fibers the second day. The fibers round up and become detached from the intermyotomal septa. By the 4th day those specimens irradiated prior to somite 1 formation (8th hour) have no functional muscles left (Fig. 29). The intermyotomal septa remain, but the spaces between them are swollen, edematous cavities containing a few wispy or rounded necrotic muscle fibers (as in Fig. 27). The muscles of embryos irradiated after this early period frequently recover, having vacuolated patches scattered through the otherwise normal muscles (Fig. 31).

An interesting phenomenon that frequently appears is a flexion of the trunk or tail (Fig. 3). This flexion is not specific to irradiation

MUSCLES

Hours of Development	*Normal Development*	*Radiation Results*
0—		} ... Virtually no muscle fibers differentiated. (Fig. 29)
5—		
	—Somite 1.	
10—	—Somites 3-4.	
	—Somites 8-9.	—(Fig. 31)
15—	—Somites 12-16.	
		} ... Excessive twisting of trunk. (Fig. 3)
20—		
25—	—Muscle fibers differentiate.	—(Fig. 27)
30—	—Muscle movement.	
	—Skeletal muscle striations.	
35—		
40—		
45—		
50—		
55—		
60—		
65—		
70—		
75—		
80—		
85—		
90—		
95—		
100—	—(Fig. 26)	

at any one stage, although it is produced more frequently by exposure from 17-19 hours. It also appears occasionally in controls, so it is not a specific result of irradiation. It may appear at any time from one day to three weeks after exposure, and does so in a relatively short time, that is, the full extent of the flexion occurs within a day. Histological examination of the region of the bend indicates muscle fiber necrosis, the extent of which is directly proportional to the period of time since the flexion occurred. It is not known whether the necrosis causes the flexion or is the result of it.

Gut Including Periblast and Pharynx

Normal Development

As cleavage proceeds, a certain amount of yolk is included within the blastomeres. The small intracellular yolk granules remain in scattered cells in all organs, being absorbed by intracellular digestion prior to cell differentiation.

After three hours of development the periblast nuclei have been formed and start differentiation, and at six hours start migrating peripherad to locations throughout the interface between the yolk and embryonic cells. Transitory Kupffer's vesicle appears at seven hours and disappears by 22 hours. At 21 hours the posterior portion of the axial endoderm begins to thicken and within one hour the hind and mid gut appear as solid rods. At 26 hours the mesenchymal visceral arch region condenses to form the solid pharynx and esophagus. This thick mesodermal pharynx-gill slit area is the most prominent organ in the body, outside of the brain, and is highly mitotic. Its proliferation stops at 54 hours when it begins to differentiate into the gill arches and other derivatives. In other teleost species (Wilson, 1889) the gut forms a cavity by a process of folding, as an inverted V, and then closes ventrally to include the lumen. In this species, however, the entire gut cavity is formed by the organization of the rod of cells into layers, with a lumen formed by the process of cavitation. This cavitation first commences in the hind and mid gut at 32 hours, and at 36 hours includes the lateral pharynx and anus. A "plug" of tissue remains just anterior to the anus which is removed at 46 hours. At 48 hours the esophagus cavitates leaving the entire gut open, except for the mouth, which opens at 90 hours. The periblast nuclei (Fig. 6) disappear as the yolk is converted into oil or is ab-

DIGESTIVE SYSTEM

Hours of Development	*Normal Development*	*Radiation Results*
0		
	—Periblast nuclei stop mitosis, start differentiation.	..... Intracellular yolk absorption inhibited.
5	—Periblast nuclei migrate peripherad.	..... Visceral arches do not differentiate.
	—Kupffer's vesicle.	
10		..... Visceral arches suppressed but still differentiate.
15		..... Gut coiling inhibited. (Fig. 31)
20	—Posterior thickening of endoderm.	..... Mucosa separates from muscularis, stomach to rectum. (Fig. 32)
	—Hind and mid gut solid rod, Kupffer's vesicle disappears.	
25	—Pharynx and esoph. solid rods.	
30	—Hind and mid gut cavitate.	
35	—Pharyng. pouch cavitate, anus opens.	
40	—Liver diverticulum.	
45	—Anal plug removed.	
	—Esophagus cavitates.	
50		
55	—Gill arches differentiate.	
60	—Gut coiling starts.	
65		
	—Stomach distends.	
70		
75		
80		
85		
90	—Mouth opens.	
	—(Fig. 30)	
95		
	—(Fig 28)	
100	—Yolk largely absorbed.	

sorbed. The yolk sac remains for several days as a distended sac, extending laterad on both sides (Figs. 2, 4). It is filled with a light oily fluid which serves as a hydrostatic organ (Peters, 1946).

Radiation Results

The undifferentiated pharyngeal region, having a high mitotic index, is the third most radiosensitive area in the unhatched embryo, following the immature blood cells and the nervous system. Exposure between two and seven hours produces necrosis and prevents differentiation into gill arches (Figs. 16, 29). After this time, up to 14 hours, differentiation is suppressed but not entirely prevented.

The periblast cells are quite insensitive to 1000 r after they have ceased proliferation at three hours (note effects of 5000 r, Fig. 7). They are probably radiosensitive prior to this, but the few survivors of very early irradiation did not permit adequate study of this.

Exposure between fertilization and the ninth hour inhibits absorption of the small intracellular yolk granules scattered throughout the body. Between 4 and 20 hours the coiling of the gut may be suppressed (Fig. 31). Exposure any time after seven hours causes a separation of the layers of the gut, between the mucosa and the muscularis, in all regions from the stomach to rectum, but not in the foregut (Fig. 32). Irradiation prior to 21 hours delays the disappearance of Kupffer's vesicle.

Heart

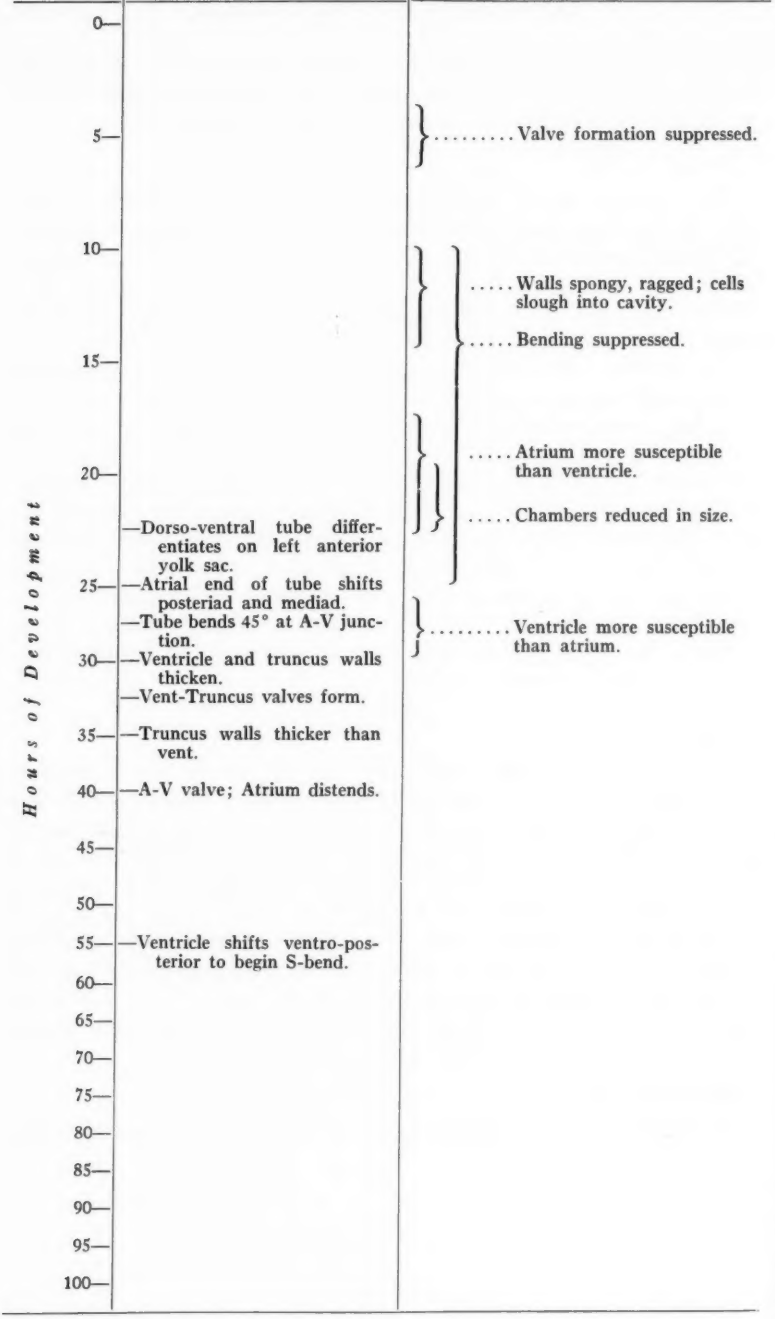
Normal Development

The heart tube is first visible at 23 hours, where it is formed as an oblique linear condensation of hemocytoblasts under the left eye along the anterior margin of the yolk sac. Differential growth brings the dorsal attachment posterio-mediad and, as the cranial flexure of the body axis occurs, the heart is found as a single ventral, pulsating tube in the body midline. At 30 hours the walls of the truncus and ventricle thicken slightly and three hours later a valve develops between them. At 40 hours the atrio-ventricular valve forms, and the atrium distends to become a thin-walled sac. At 54 hours the ventricle shifts posterio-ventrad, commencing the typical S-bend, which is completed in its adult form at about two weeks.

Radiation Results

As shown in the accompanying chart, the heart displays six types

HEART


Normal Development
Radiation Results
Hours of Development
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
—Dorso-ventral tube differentiates on left anterior yolk sac.
—Atrial end of tube shifts posteriad and mediad.
—Tube bends 45° at A-V junction.
—Ventricle and truncus walls thicken.
—Vent-Truncus valves form.
—Truncus walls thicker than vent.
—A-V valve; Atrium distends.
—Ventricle shifts ventro-posterior to begin S-bend.
Valve formation suppressed.
Walls spongy, ragged; cells slough into cavity.
Bending suppressed.
Atrium more susceptible than ventricle.
Chambers reduced in size.
Ventricle more susceptible than atrium.

of specialized sensitivity to irradiation, in addition to a general lag and size reduction following pycnosis and necrosis. Exposure during 4 to 6 hours of development suppresses subsequent valve formation. Exposure from 10-13 hours produces heart walls that appear ragged and "spongy," with cells sloughing into the cavity. Irradiation at any time from 10-26 hours suppresses subsequent bending of the heart. Exposure during the 20-23 hour period reduces the size of the chambers. The atrium is more susceptible than the ventricle from 17-23 hours, whereas the ventricle is more suscepticle than the atrium at 27-29 hours. A generalized result of irradiation at all stages is a breakdown of the endocardium layer. It cannot be detected whether this occurs in the blood vessels also, but this may be responsible for the generalized edema which so frequently follows irradiation (Figs. 5, 23, 29, 31).

No effect on heart beat could be detected. It continues to beat even when in a state of partial necrosis. Perhaps the autonomous, cardiogenic nature of the stimulus is responsible for this.

Ear-Lateral Line Complex

Normal Development

As the neural tube is formed a group of cells is located lateral to the brain. This is the neural crest (or cranial crest), and becomes distinct as the otic primordium at 12 hours. The anterior portion is continuous with the inner (nervous) layer of ectoderm. At about 15 hours the posterior portion of this mass begins to condense and vesiculate, forming the otic capsule. In contrast to other vertebrate groups, the otocyst does not form a distinct placode and invaginate as a discrete group of cells, but the hollow vesicle forms from the loose, mesenchymal cells. At 19 hours the anterior portion of this cranial crest mass, at its point of fusion with the nervous ectoderm, forms columnar cells and becomes a lateral line organ. The posterior lateral line anlagen begin to differentiate at about 33 hours. They form *in situ* from the nervous layer of ectoderm, and histologically resemble the maculae of the ear.

Radiation Results

The formation of the ear is suppressed by irradiation during the period of 7 to 13 hours. The condensation of the cells often does

EAR — LATERAL LINE COMPLEX

Hours of Development	*Normal Development*	*Radiation Results*
0		... Lag in chamber formation.
5		
10		... Suppression of all ear structures.
	—Otic primordia distinct.	
	—Partial delamination from ectoderm.	
15	—Otic primordia condense.	
	—Otic capsule vesiculates.	
	—Lat. line anlagen post. to ear.	
20	—Otic vesicle detaches from ectod.	
25		... Capsule contours irregular.
30	—Otic ganglia formed.	
	—Endolymphatic duct formed, flank lat. line differentiates.	
35	—Maculae and semicircular canals.	
40		
45		
50	—Partitions begin to form.	
55		
60	—Ear 3-chambered.	
65		
70		
75		
80		
85		
90		
95		
100		

not occur. At 24-25 hours the development of the capsule may be suppressed, resulting in a hollow sphere of cells which remains only one cell layer thick, and does not further differentiate.

Pronephros

Normal Development

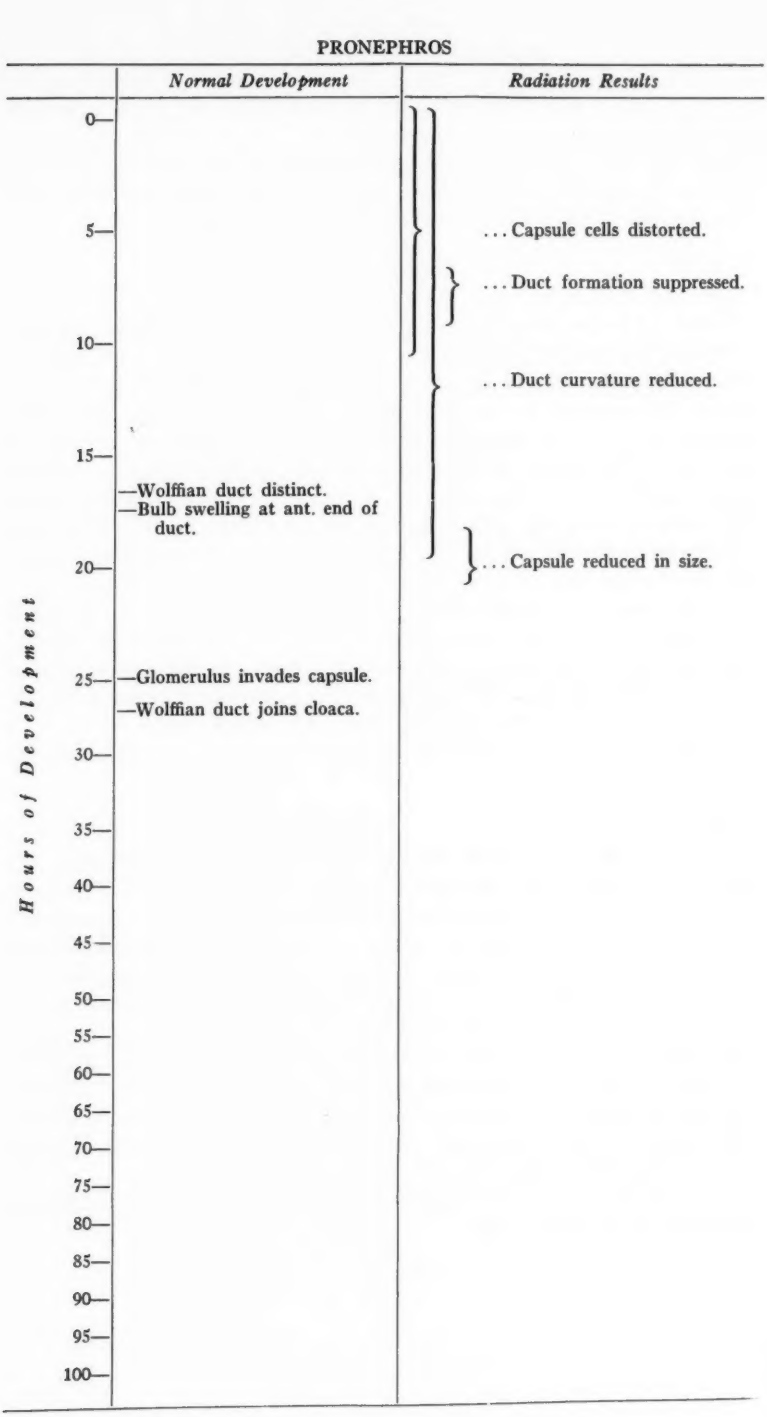
About 17 hours after fertilization, shortly after completion of gastrulation, the paired Wolffian ducts begin to visibly differentiate as loosely arranged segments of undifferentiated cells opposite each somite, dorso-lateral to the gut. Within an hour these segments unite linearly and the cells become cuboid, arranged around the lumina of the ducts. The ducts form in the shape of question marks, curving laterad nearly to the edge of the body just posterior to the otic vesicle. The anterior ends are enlarged, bulb-like swellings that lie just ventro-lateral to the notochord. At 25 hours a tuft of capillaries pushes into the medial side of each vesicle, forming the glomerulus and capsule. At about 27 hours the posterior ends of the ducts join the temporary cloaca, and later in development the atriopore is formed separately from the anus as the external opening of the genito-urinary systems. After about one month the mesonephric tubules begin to form, and by two months they are large, functional organs.

A large mass of hematopoietic tissue surrounds each capsule from its earliest stages.

Radiation Results

The Wolffian ducts tend not to differentiate after irradiation between 7-9 hours after fertilization. The curvature of the duct is markedly reduced by irradiation within the first 20 hours; the earlier the exposure the less the curvature. Prior to 7-8 hours irradiation tends to suppress all curvature; at 7 or 8 hours, if it does not prevent the formation of the duct, it reduces its subsequent curvature to an L-shape; exposure within a few hours after this produces a J-shaped duct. After about 20 hours irradiation does not affect the curvature. Bowman's capsule is sensitive at two periods: prior to 10 hours irradiation produces a capsule of normal size but made up of abnormal, distorted cells; exposure between 18-21 hours causes a small but histologically normal capsule.

The hematopoietic elements of the pronephros are completely destroyed in the early stages.


PRONEPHROS
Normal Development
Radiation Results
Hours of Development
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
—Wolffian duct distinct.
—Bulb swelling at ant. end of duct.
—Glomerulus invades capsule.
—Wolffian duct joins cloaca.
... Capsule cells distorted.
... Duct formation suppressed.
... Duct curvature reduced.
... Capsule reduced in size.

Olfactory Organs

Normal Development

The olfactory organs first become visible about 20 hours after fertilization as a placode of cells in the nervous layer of ectoderm. Within 2-3 hours the nerves establish connection with the adjacent forebrain. The cells become columnar and a pit invaginates into the center from the outside (Fig. 24).

Radiation Results

The sensitive period is limited to 14-18 hours after fertilization, at which time there is not yet visible histodifferentiation. Exposure during this period results in pycnosis of cells, followed by necrosis and dedifferentiation of cells. The relatively high radioresistence before and after this period of sensitivity is demonstrated, in Figs. 16 and 25, by the fairly normal morphology of the olfactory organ, in comparison to the brain and eye. Fig. 16 shows the results of 1000 r at 3 hours; Fig. 25 shows the result of 5000 r at 19 hours. There is no cavity into which the necrotic cells slough and they remain in place until death or until they are dissolved and replaced in event of recovery. The invagination process is inhibited or stopped by the cellular dedifferentiation.

Notochord

Normal Development

The notochord is first discernable at 9 hours as a loose group of cells ventral to the posterior keel. This loose organization proceeds anteriorly until, at 13 hours, the anlage is complete from head to tail. At 16 hours, commencing in the tail region, the notochord cells condense to form a rod. The cells become columnar at 20 hours and, at 25 hours, have commenced vacuolation. The larval notochord is completely differentiated by 39 hours.

Radiation Results

The notochord is sensitive to irradiation only during the 4th to 7th hours. Exposure during this period reduces the subsequent size or completely suppresses the formation of the notochord. In many irradiated specimens the dorsal surface of the notochord is wrinkled, but this is thought to be due to shrinkage of the more sensitive adjacent neural tube.

OLFACTORY ORGANS

Hours of Development	*Normal Development*	*Radiation Results*
0		
5		—(Fig. 16)
10		
15		...Necrosis and dedifferentiation of placode.
20	—Olfactory placode first visible.	—(Fig. 25)
25		
30		
35	—(Fig. 24)	
40		
45		
50		
55		
60		
65		
70		
75		
80		
85		
90		
95		
100		

NOTOCHORD

Hours of Development	*Normal Development*	*Radiation Results*
0		
5		...Notochord formation suppressed.
10	—Notochord anlage organized at caudal end.	
15	—Anlage complete from head to tail. —Cells condense to form a rod, commencing at tail.	
20	—Cells become columnar.	—(Figs. 5, 14)
25	—Cells commence vacuolation.	
30		—(Fig. 27)
35		
40	—Larval notochord differentiated.	
45	—(Fig. 13)	
50		
55		
60		
65		
70		
75		
80		
85		
90		
95		
100		

Pigment Cells Chromatophores

Normal Development

Chromatophores originate in the neural crest (Rawles, 1948). The stages at which they become separated from the parent material and migrate to their respective positions have not been determined for this species, nor for teleosts in general (Tavolga, 1949.) Primitive chromatophores are indistinguishable from the mesenchyme cells. They appear to be *in situ* at the time the pigment granules make their first appearance at 17 hours. They appear simultaneously in several locations in the body wall, especially around the yolk sac, and surrounding the nerve cord in the trunk. Pigment appears in the retina at 29 hours, in the sclera at 35 hours, and in the iris at 60 hours. In other teleost species melanin first appears in the retina.

Radiation Results

In this experiment the chromatophores and their precursors prove to be very insensitive to irradiation. No response to 1000 r can be detected at any stage from fertilization to hatching. Slight pycnosis is noted about 30 hours after exposure to 5000 r at the 19th hour (Fig. 14, 25). No effect upon differentiated chromatophores can be demonstrated, although it has been reported elsewhere (Rugh, 1949) that irradiation produced darkening in tadpole larvae.

Discussion

Normal Development

Certain features of the development of the Paradise Fish present interesting departures from the typical teleost or vertebrate patterns.

The otic vesicle in all other teleosts, as reported in the literature, develops as an invagination of a solid placode formed in the inner, nervous layer of the ectoderm. In this species a placode is formed, but it is posteriorly in continuation with a mass of condensing cranial crest material. The placode and cranial crest cannot be distinguished from each other in the resulting mass. The posterior portion of the mass condenses, and a lumen appears in the center which becomes the otocyst. The anterior portion of the mass (the placode) separates from the otic vesicle and forms a portion of the lateral line organ.

The typical teleost pattern of gut formation was described in the Salmon by Wilson (1889) and by Mahon and Hoar (1956) as a

process of folding as an inverted V, enclosing the lumen of the gut by ventral closure of the folds. Price (1934) described a modified pattern in the pharynx and esophagus of the Whitefish in which the gut forms as a solid rod in which a cavity develops *de novo*. The entire gut of the Paradise Fish forms in this latter manner.

Another unique feature of this species is that the heart tube first forms, not in the mid-line of the body, but laterad, on the anterior margin of the yolk sac under the left eye. The heart later moves into the mid-line. This same phenomenon has been reported in another Anabatid (Ingersoll, 1951) as well as in one of the Gasterosteidae (Swarup, 1958).

Radiation Effects

The most significant conclusions to be drawn from the experiments described in this paper concern the changing patterns of differential susceptibility in the various organs and tissues and the localization in time of the "critical periods." Work done on this problem in the first half of the present century has been summarized by Child (1941). Russell's work (1956) on the mouse is an excellent example of some of the recent work. The present paper adds data from a class of organisms whose radiobiological responses are not well known (O'Brien 1956). In the present experiment the abnormalities that have been induced are not unique to the species nor to the experimental procedure. Many of the induced deformities have also been found in controls. They are considered to be responses to nonspecific stimuli which interfere with normal developmental processes.

As each tissue or organ begins a period of high mitotic activity it rises in sensitivity to irradiation. Metabolic activity and differentiation may also be correlated with this susceptibility. Certain tissues, at the height of their sensitivity, are more or less resistent than other tissues at the height of their sensitivities. These differences may be due to the mitotic index, metabolic factors, or, perhaps, to unknown "intrinsic" factors.

During the first four days of development, the embryonic tissues may be listed approximately in the following order of their decreasing sensitivity:

1. Hematopoietic tissue
2. Eye
3. Optic lobe of brain
4. Posterior nerve cord
5. Central nervous system (other than above)
6. Pharynx
7. Immature germ cells
8. Muscle
9. Olfactory organ
10. Gut
11. Heart
12. Excretory system
13. Notochord
14. Chromatoblasts
15. Cartilage
16. Differentiated periblast (the sensitivity of immature periblast is unknown)
17. Mature erythrocytes

The various organs and tissues differ somewhat in their abilities to recover from radiation damage. This is thought of, in part, as an expression of their regulatory and mosaic properties. An example may be drawn from the variable responses of different portions of the eye. The inner layers of the retina promptly displayed intensive injury after irradiation while cellular proliferation was proceeding; in some cases these layers were almost completely destroyed. Yet after four days the area appeared completely normal in almost every case. Another part of the eye, the rod and cone layer of the retina, did not manifest much damage for two or three days after exposure, but when damage did occur it persisted for several days as breaks in that layer. The inner layers of the retina were highly mitotic during and after exposure. The mitotic process is especially sensitive to irradiation and therefore many cells were promptly damaged. The mitotic activity continued in the undamaged cells, however, and the necrotic cells were promptly replaced. On the other hand, the rod and cone layer of the retina had little mitotic activity after the initial period of proliferation. These rather inert cells displayed no damage after irradiation until the time arrived for differentiation of the rod and cone elements. Since there was little mitotic activity, damage of this type was not readily reparable.

Thus, tissues with a high mitotic index are easily damaged but quickly recover. Differentiating tissues with a low mitotic index are less susceptible to damage and are correspondingly less capable of recovery. This may be a part of the answer to the apparent regulatory and mosaic features of developing embryos (Waddington, 1956).

Summary

Embryos of the Paradise Fish were exposed to x-rays at different stages of development. Histologic studies were made of several selected organ systems in order to determine the stages at which par-

ticular anomalies might be induced. In each organ system certain anomalies were characteristically produced by irradiation at specific developmental stages. In most cases the abnormalities were induced by exposure prior to visible differentiation of the anlagen of the affected structures. Both susceptibility to radiation damage and the ability to regenerate from the damaged condition are directly proportional to the mitotic index of the tissues at the time of and following exposure. Differentiation processes are also susceptible to damage but, when unaccompanied by active mitosis, have less regenerative capacity. Some notes on normal development are also presented. *Macropodus* differs from the "typical" teleostean patterns of development in the ear and the gut. The otocyst arises by cavitation of a cranial crest mass which is attached to neural ectoderm. The gut lumen forms by cavitation of a longitudinal rod of cells rather than by ventral folding of an entodermal plate.

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PLATE I

Explanation of Figures

Figure 2—Control, 6 days after hatching. 25x

Figure 3—Irradiated with 1000 r at 16 hours, fixed 6 days after hatching. Note absence of left eye. 25x

Figure 4—Control, 4 days after hatching. Cross section at level of yolk sac, swim bladder, intestine, liver, and pancreas. 70x

Figure 5—Irradiated with 1000 r at 20 hours, fixed 4 days after hatching. Cross section as in Fig. 4. Note undifferentiated gut, liver, and swim bladder; edema in coelom and yolk sac; decayed muscles. Spinal cord is small but histologically nearly normal. 70x

Figure 6—Control one day after hatching. Section through edge of yolk sac, showing well-differentiated periblast nucleus. Periblast cytoplasm appears as fine granules around nucleus. 700x

Figure 7—Irradiated with 5000 r at 19 hours, fixed one day after hatching. Section through edge of yolk sac showing undifferentiated periblast nuclei. 700x

Figure 8—Control, 20 hours. Section through retina. Mitotic figure at upper right. 700x

Figure 9—Fixed immediately after irradiation with 5000 r at 19 hours. Section through retina, showing increased number of mitotic figures. Note bridge formation. 700x

Figure 10—Fixed one hour after irradiation with 5000 r at 19 hours. Section through retina. Note pycnosis, absence of mitotic figures. 700x.

Figure 11—Fixed 8 hours after irradiation with 5000 r at 19 hours. Section through retina. Note pycnosis. 700x

Figure 12—Fixed 24 hours after irradiation with 1000 r at 14 hours. Section through forebrain. Note damage in optic lobe (above) compared to rest of forebrain. 315x

Figure 13—Control, 45 hours, nine hours after hatching. Section through myelencephalon and otic capsule. 315x

Figure 14—Fixed at same age as Fig. 13. Irradiated with 5000 r at 19 hours. Note extreme degeneration of neural tube, extrusion of pycnotic debris into neurocoele, relatively unaffected notochord and pigment cells.

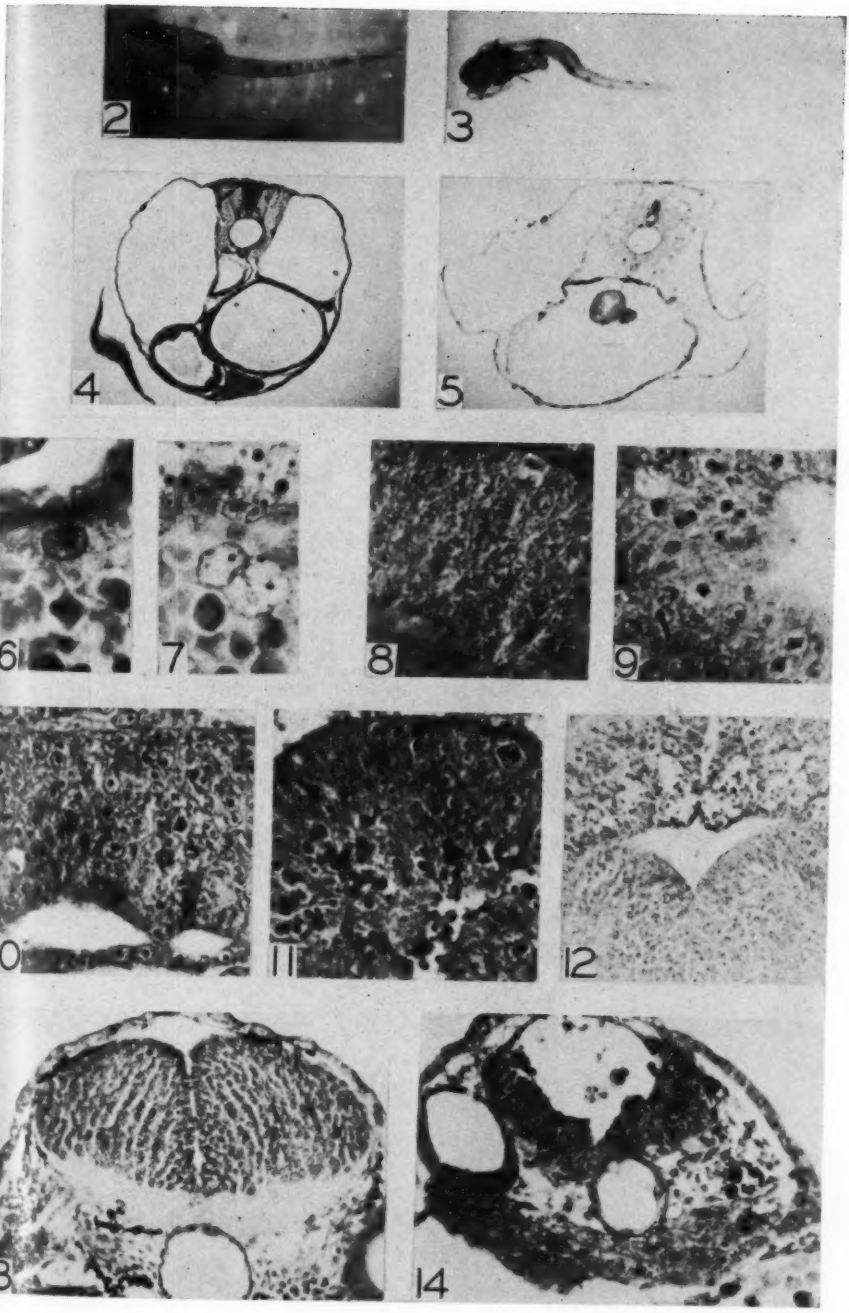
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PLATE II

Explanation of Figures

Figure 15—Control, 4 days after hatching. Cross section through eyes, gills, pharynx, and diencephalon. 70x

Figure 16—Irradiated with 1000 r at 3 hours, fixed 4 days after hatching. Section as in control, Fig. 15. Note degeneration of eyes and brain, lack of differentiation of brain and pharynx. 70x

Figure 17—Control, 38 hours, 2 hours after hatching. Section through eye. 315x

Figure 18—Irradiated with 1000 r at 27 hours. Fixed 2 hours after hatching (12 hours after exposure). Section through eye. Note pycnosis. 315x.

Figure 19—Control, 4 days after hatching. Section through eye. 315x

Figure 20—Irradiated with 1000 r at 20 hours, fixed 4 days after hatching. Frontal section through eyes, mid brain, pharynx. 70x

Figure 21—Enlargement of left eye in Fig. 20. Note degeneration of retina, especially of rod and cone cells. 315x

Figure 22—Control, one day after hatching. Cross section through eyes, midbrain, pharynx. 70x

Figure 23—Irradiated with 5000 r at 19 hours. Fixed simultaneously with control. Section through eyes and gut. Note degeneration and/or lack of development in all head structures; edema. A small portion of the gut is shown because of ventral flexure. 70x

Figure 24—Control, at time of hatching. Section through snout to show olfactory organ, eye, brain. 315x

Figure 25—Irradiated with 5000 r at 19 hours, fixed 18 hours later (Simultaneously with control, Fig. 24). Section as in control. Note degeneration and lack of differentiation of head structures. 315x

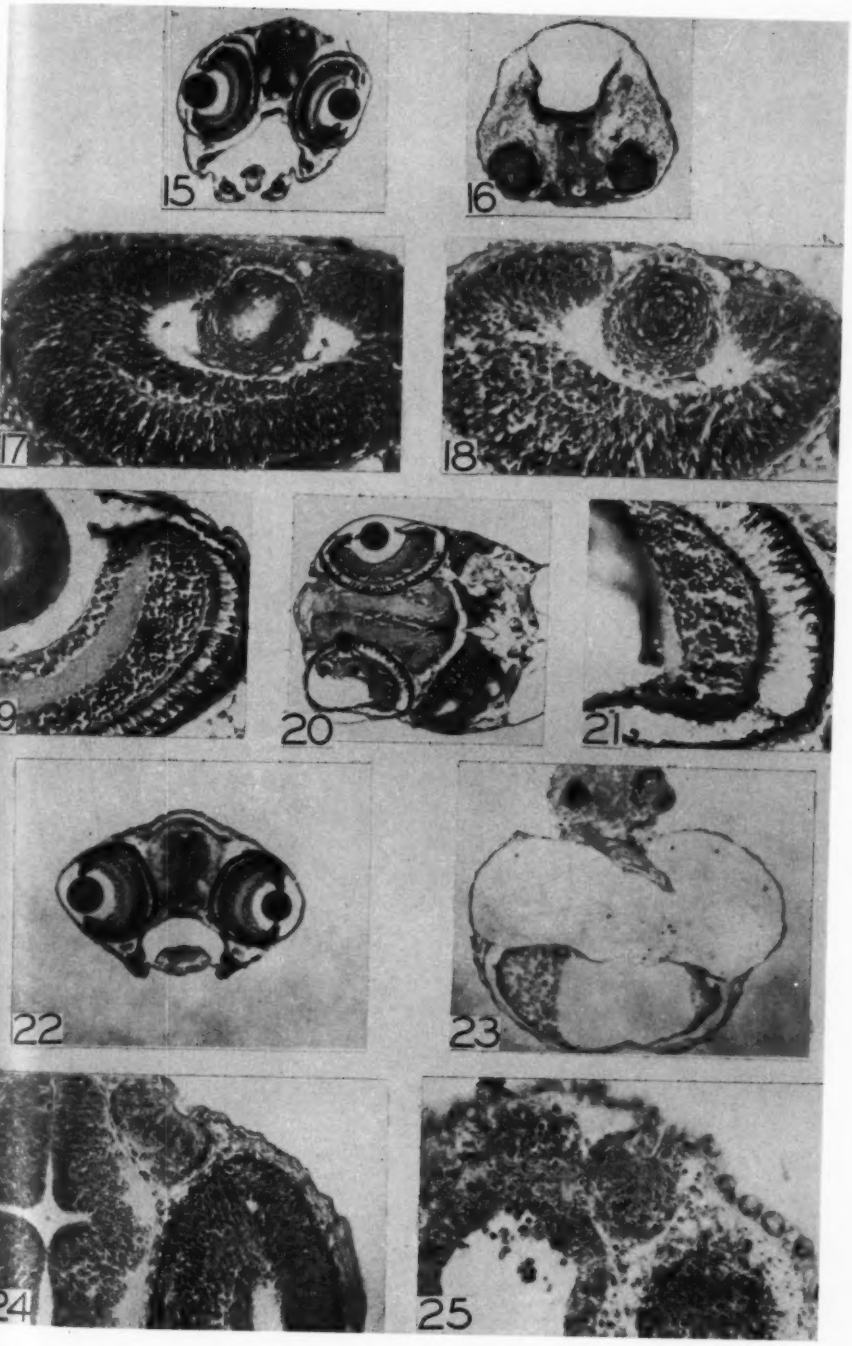
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PLATE III

Explanation of Figures

Figure 26—Control, 99 hours, 63 hours after hatching. Longitudinal section through tail muscle. 315x

Figure 27—Irradiated with 1000 r at 28 hours, fixed and sectioned as in control, Fig. 26. Note degeneration of fibers, persistence of intermyotomal septa. 315x

Figure 28—Control, 98 hours, 62 hours after hatching. Cross section through gill arches. 70x

Figure 29—Irradiated with 1000 r at 3 hours, fixed and sectioned as control, Fig. 28. Note lack of differentiation of gill arches, brain, notochord, and pharyngeal muscles. 70x

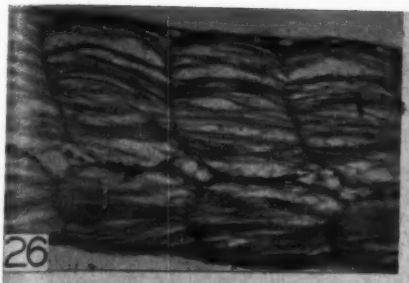
Figure 30—Control, 91 hours, 55 hours after hatching. Frontal section at level of digestive tract. Three cross sections through intestine indicate coiling. 70 x

Figure 31—Irradiated with 1000 r at 12 hours. Fixed 8 hours earlier than control, Fig. 30. Oblique saggital section through gut, eye. Note absence of coiling in gut, edema, degeneration of eye, ear, and muscles.

Figure 32—Irradiated with 1000 r at 20 hours, fixed at 91 hours, 55 hours after hatching. Section through gut, showing retraction of mucosa from outer layers. 315x

Figure 33—Control, 19 hours after fertilization. Section through hematopoietic mesenchyme on ventrolateral edge of yolk sac. 1200x (Photo courtesy of Zane Price)

Figure 34—Irradiated with 1000 r at 1 hour after fertilization, fixed 12 hours later. Section through presumptive hemocytoblasts at edge of yolk sac. Note pycnotic mesenchyme cells. 1000x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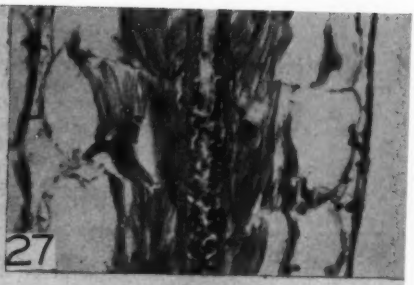
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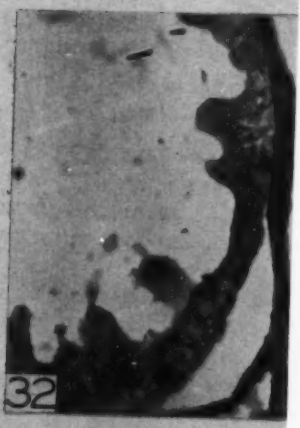
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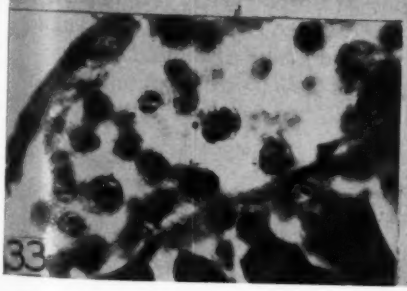
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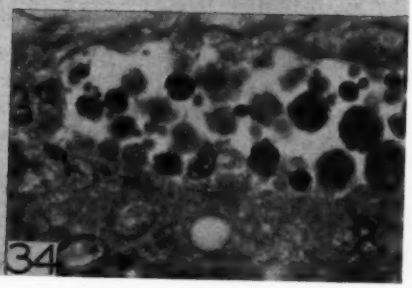
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EXPERIMENTAL PRODUCTION OF CHRONIC THYROIDITIS

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Chronic thyroiditis has been produced in experimental animals by various methods (1, 2, 3, 4). The problem of its etiology and histogenesis, however, remains unsolved (5).

Recently Witebsky and coworkers (6) injected thyroid extract into the skin of animals and several weeks later observed a severe inflammation of the thyroid. They explained this as autoimmunization. They could demonstrate the presence of antibodies against thyroglobulin in the injected animals, and they believed that these immune bodies reach the thyroid and by combining with thyroid colloid elicit inflammation as an antigen-antibody reaction.

The following experiments were undertaken to find out whether substances other than thyroid extract, after intradermal injection, may produce chronic thyroiditis.

Experimental Studies

Forty-two guinea pigs weighing between 500 and 700 grams were divided into five experimental and three control groups. All animals were kept in separate metal cages under utmost cleanliness. The basic diet consisted of Purina Fox Chow and tap water supplemented with fresh lettuce and carrots.

Various drugs were injected intradermally into the depilated anterior abdominal wall. All experimental animals, except one in Group II, and all controls survived the experimental periods and were killed in Nembutal anesthesia by exsanguination. The sites of injection and the thyroids of all animals were fixed in formalin and in dichromate of mercury. They were then embedded in paraffin and sectioned six microns thick. The paraffin sections were stained with hematoxylin and eosin and with Sudan black in glycol.

In the first experiment, 5 grams of thyroid tissue obtained from a fresh surgically removed colloid goiter was homogenized in a tissue

TABLE I

Experiments	Number of Animals	Intradermal Injection	Duration	Inflammation of Skin	Thyroid
1	4	Aceton extract of nodular colloid goiter.	6-21-58 to 8-29-58	+++	Colloidophagy in 4-6 follicles. Lymphocytic foci : 6-14.
2	8	Desiccated thyroid (Parke Davis & Co.) emulsified in Freund Adjuvant	11-24-58 to 12-29-58	+++	Colloidophagy in 4-10 follicles. Lymphocytic foci : 6-12.
3	4	I^{131} labeled Triolein	10-2-59 to 10-9-59 and 10-16-59	++	Colloidophagy in 3-4 follicles. Lymphocytic foci : 2-3
4	5	Pantopaque in Freund Adjuvant	2-21-59 to 4-4-59	+++	Colloidophages in 3-5 follicles. Lymphocytic foci : 4-11.
Controls					
A	11	No injection.	11-29-58 to 12-29-58	Neg.	Negative
B	5	Freund Adjuvant	2-7-59 to 3-23-59	+++	Negative
C	5	Defatted thyroglobulin in Freund Adjuvant	8-8-59 to 9-19-59	+++	Negative

grinder with physiological salt solution. The homogenate was extracted with 10 volumes of cold acetone. After centrifugation the supernatant fluid was evaporated to dryness at room temperature, and the residue was suspended in physiological salt solution. Chemical analysis showed that the lipid preparation contained 265 micrograms of iodine per cc.

Four guinea pigs were injected intradermally with 0.1 cc. of this emulsion. After three days a hard swelling developed at the site of injection and persisted for six weeks, until the end of the experimental period.

Microscopic examination of the skin showed a severe inflammatory reaction. Many macrophages containing lipoid material were present in addition to lymphocytes and multinuclear giant cells. The macrophages in certain parts of the sections surrounded masses of the injected lipoid material. In the periphery of the inflammatory area there were many fibroblasts.

The thyroid showed evidence of chronic thyroiditis. In every section there were from four to six follicles containing macrophages in their lumina and many groups of lymphocytes were present in the adjacent stroma.

For the second experiment eight guinea pigs were used. We prepared an emulsion of 2 grains of desiccated thyroid (Parke, Davis & Co.) in 1 cc. of Freund Adjuvant. Microscopic examination of finely powdered material of the thyroid tablets showed flakes of colloid which stained intensively with Sudan, and chemical analysis proved a 1.8 per cent content of lipid.

Freund Adjuvant (Difco Laboratories, Detroit) is a mixture of mineral oil, killed *mycobacterium tuberculosis* and unsaturated mannite monoleate. We injected intradermally 0.1 cc. of the Thyroid-Freund Adjuvant emulsion and after a few days observed an inflammatory tumor about 1 cm. in diameter at the site of injection.

Microscopic examination of the skin six weeks after injection showed a severe granulomatous inflammation with large groups of mononuclear cells. There were many round spaces filled with lipid surrounded by giant cells and lymphocytes. In the thyroids of all animals we saw from six to twelve large foci of lymphocytic infiltration, and 6 to 10 follicles contained macrophages in each section.

In the third experiment we injected four animals with I^{131}—labeled

FIGURE 1

Lipogranulomatous inflammation in skin of guinea pig 6 weeks after intradermal injection of Pantopaque (iodinated protein-free lipid. Fat-filled spaces surrounded with macrophages, lymphocytes, plasma cells and fibroblasts (× 450).

FIGURE 2

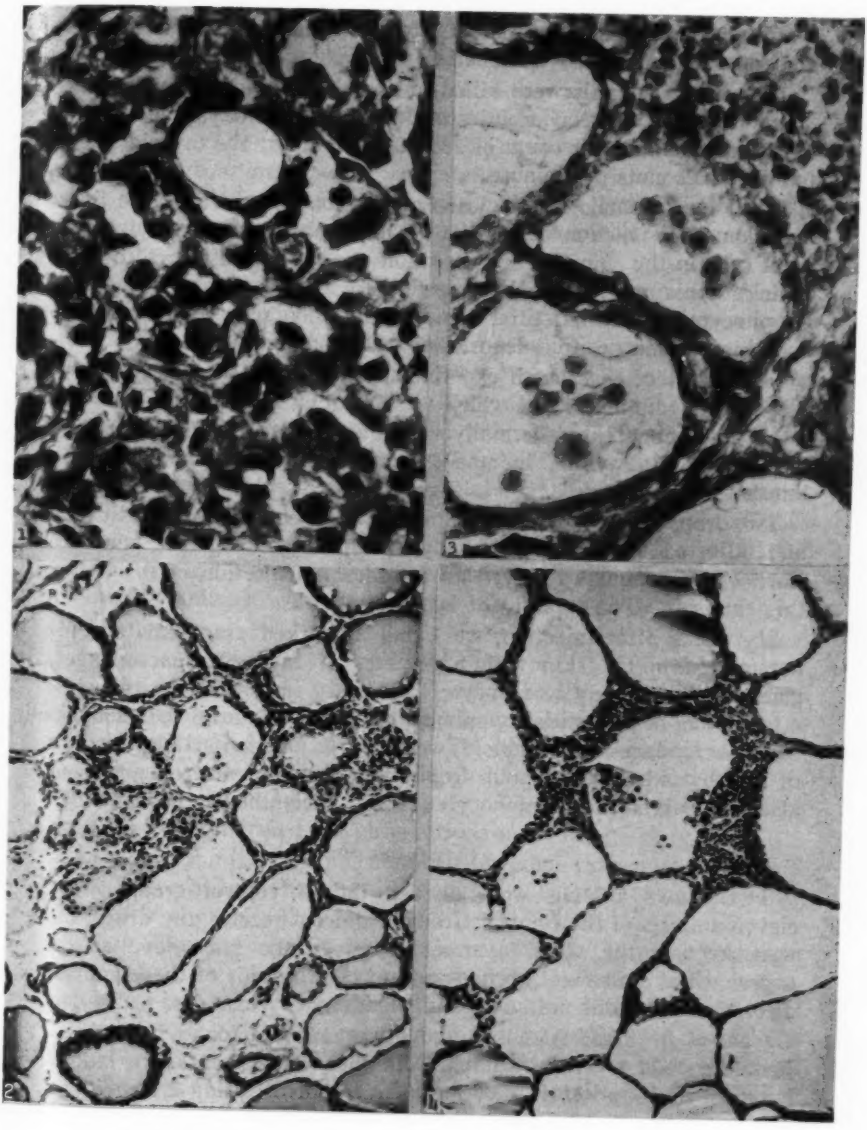
Thyroiditis in the same animal. In 2 follicles are macrophages, the colloid is resorbed and lymphocytes have accumulated in the adjacent stroma (× 150).

FIGURE 3

Same thyroid after staining with Sudan black for lipid. Macrophages in 2 follicle lumina contain small globules of lipid. In the adjacent inerstitial tissue lymphocytes surround released droplets of lipid.

FIGURE 4

Chronic thyroiditis 6 weeks after intradermal injection of thyroid extract emulsified in Freund Adjuvant. Groups of macrophages are noticed in 2 follicles. There is marked lymphocytic infiltration of the surrounding interstitial tissue.



Triolein (Abbott Laboratories, Chicago) which is a Trioleate of glycerol containing 116 microcuries of I^{131}.

Two of the animals were killed one week after injection and the others fourteen days after injection. After seven days the site of injection gave a Geiger count of 25,000 per minute; the thyroid registered 4,500 counts per minute.

In all four animals histological examination showed a severe lipogranulomatous reaction with fat-laden macrophages, lymphocytes and giant cells in the skin. In every section of the thyroids three to four follicles contained macrophages, and there were as many groups of lymphocytes in the interstitial tissue. Autoradiographs of the thyroids and skin showed positive deposits of radio-iodine.

The object of the fourth experiment was to investigate the effect of another iodinated lipoid which was free of thyroglobulin. We injected five animals intradermally with Pantopaque (LaFayette Pharmacies Inc., LaFayette, Indiana). This is Ethyl-Iodophenylundecylate and is protein free.

Two drops of Pantopaque were mixed with 5 cc. of Freund Adjuvant and adjusted until chemical analysis showed 135 μgms I^2 per cc. Each of five animals received an intradermal injection of 0.1 cc. of this emulsion. The experiment lasted six weeks.

Six weeks after injection, we found a marked granulomatous inflammation in the skin with aggregates of fat-laden macrophages, giant cells and many lymphocytes. In every section of the thyroids, 4 to 11 foci of interstitial lymphocytic infiltration and 3 to 5 follicles with macrophages in the colloid, were noticed. Sudan stained sections of the thyroid showed lipoid droplets, not only in macrophages, but also within groups of lymphocytes in the interfollicular tissue.

Controls

Twenty-two animals were divided into 3 control groups. The eleven animals of the Control Group A did not receive any drugs, but were kept in the same room and received the same food as the experimental animals. Careful study of the thyroids of these animals after six weeks did not show any inflammatory changes.

Control B. Since Witebsky and coworkers had found Freund Adjuvant such a powerful stimulant in their experiments, we studied its effect by intradermal injection in five animals, using 0.1 cc. After

six weeks the site of injection showed an intensive granulomatous reaction, but the thyroids of all five animals were entirely normal.

Control C. Crude thyroid extract with which Witebsky and coworkers had succeeded in producing chronic thyroiditis after intradermal injection contains considerable amounts of iodinated lipid. We therefore studied the effect of an almost completely defatted thyroid preparation which we had obtained from R. L. Kroc, Ph.D., Director of Physiological Research, Warner-Lambert Research Institute, Morris Plains, N. J.

This thyroglobulin had been prepared by grinding frozen hog thyroids followed by extraction with neutral 1 per cent aqueous saline at 5° C. It was filtered in a pre-cooled filter. The major aliquot was acidified and heated to precipitate proteins which were then extracted with hot alcohol to remove water and residual lipid. The chemical analysis showed 1.02 per cent total iodine, 0.314 per cent thyroxin iodine (Blau 1955) and 0.58 per cent Soxhlet alcohol extractibles.

Two grains of this defatted thyroglobulin were emulsified in 1 cc. of Freund Adjuvant, and of this emulsion each of the five animals received an intradermal injection of 0.2 cc. After six weeks, all five animals showed a granulomatous reaction in the skin, but the thyroids of the five animals were entirely free of inflammation.

Comment

In 1933 Ferguson (7) injected thyroid extract subcutaneously into three guinea pigs and observed severe inflammatory changes at the site of injection within 24 hours. This important biological proof, that thyroid colloid is a powerful inflammatory agent outside of its follicle confines, remained completely overlooked in the literature until we (8) confirmed his observations in 1956.

Kracht (9) injected lipid, obtained by ether extraction of thyroid tissue, directly into the thyroid of guinea pigs and observed severe thyroiditis after only two days.

When Witebsky and coworkers injected a crude thyroid extract into the foot pads of rabbits, they were primarily interested in the formation of iso-immune bodies against thyroglobulin. They were surprised to find in these immunized animals inflammatory changes in the thyroids. They assumed that antibodies against the injected thyroglobulin reach the thyroids of the experimental animals, combine

with thyroid colloid and elicit a chronic thyroiditis as antibody-antigen reaction.

While our own experiments confirm the results of Witebsky and coworkers, namely that crude thyroid extract after intradermal injection causes thyroiditis, their immunological hypothesis is not supported by our findings.

We could demonstrate that thyroglobulin by itself, without associated lipid, is ineffective in producing thyroiditis; on the other hand iodinated lipid, without thyroglobulin or any other protein, is just as effective as crude thyroid extract in producing chronic thyroiditis after intradermal injection.

Our experiments suggest the following course of events: At the site of injection a lipogranulomatous reaction results from deposits of iodinated lipid. The latter is engulfed by macrophages which are carried by the blood stream to the thyroid. Here, due to its iodine content, the lipid is avidly taken up by the thyroid tissue and is secreted into the follicle lumen. The abnormal lipid-rich colloid attracts macrophages which engulf it and carry it into the interstitial tissue. There they release their storage products. The liberated lipid-rich colloid attracts lymphocytes and plasma cells which produce antibodies. The immune bodies, therefore, are the effect, not the cause of thyroiditis.

Conclusions

Intradermal injection of iodinated lipid produces lipogranuloma in guinea pigs at the site of injection, followed by chronic thyroiditis.

This thyroiditis cannot be explained as an immune reaction against thyroglobulin because iodinated lipid is effective whether thyroglobulin is present or not.

From the site of injection, the iodinated lipid is carried to the thyroid and secreted into the lumen of follicles. It is engulfed by macrophages and carried into the surrounding stroma where it attracts lymphocytes and antibody-forming plasma cells.

Our experiments suggest that chronic thyroiditis is a defense reaction against iodinated lipid and that immune bodies are not the cause but the effect of thyroiditis.

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Growth, 1960, **24**, 179-192.

DIFFERENTIAL GROWTH IN THE SKULLS OF TWO SUBSPECIES OF DEERMICE[1]

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One determinant of adult morphology is differential growth (Thompson, 1917). The differential development of the nervous system may also contribute to adult behavior. A rapidly developing pattern of behavior may inhibit or enhance the development of a related behavioral pattern. The interaction among behavioral patterns with different rates of development could be particularly important in molding adult behavior because the sequence of development may determine the reinforcing properties of the environment.

The test of the hypothesis that differential growth affects adult behavior has been undertaken with a study of two subspecies of deermice (*Peromyscus maniculatus*) which mature at different rates (King, 1958). The purpose of the present paper is to describe differential growth in the cranial and facial bones of the skull and the brian weigths of *P. m. bairdii* and *P. m. gracilis*. A histological and biochemical analysis of the developmental changes in the brains has been initiated (Eleftheriou, 1959) and several studies on the development of behavior have been completed (King, 1958; King & Shea, 1959). After differential growth in morphology, physiology, and behavior have been clearly demonstrated, experimental procedures can be initiated in an effort to manipulate the differential growth.

MATERIALS AND METHODS

Skulls and brains of *P. m. bairdii* and *P. m. gracilis* were obtained from a colony which had been bred in the laboratory for a period of 10 to 12 years. Over 350 mice were examined from birth until 30 days of age. Approximately half were used for an analysis of the

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skull bones and the other half were used for obtaining brain weights. Actual numbers for each measurement are shown in the results (Table 1).

Although specimens were collected at many daily intervals, only those collected at birth, 5, 10, 15, 20, 25, and 30 days of age are presented in the table. Litters of 3 to 6 young were used and the entire litter was sacrificed at the designated age, calculated from within eight hours of actual birth.

The bones were collected by skinning the mice, severing the head and limbs from the body, and immersing them in boiling water for three minutes. They were then placed in vials with papaine and salt water and left overnight in an incubator at 47° C. After incubation, the bones were washed with water, bleached with hydrogen peroxide and degreased with acetone. This process separated the skull bones of the young mice. Camera lucida drawings were made of the femur, humerus, basioccipital, frontal, nasal, and parietal. The drawings were then measured. A one to three percent error was recognized in these measurements.

Brains were removed by cutting through the dorsal part of the skull and lifting them out with forceps. They were immediately weighed to the nearest milligram on a Roller-Smith torsion balance. Body weights were taken with a triple beam balance immediately after death.

The measurements taken were the greatest length of the femur and humerus, length of nasal, projective frontal length, asterion-pterion and lambda asterion of the parietal bone and the midsaggital plane of the basioccipital. For an analysis, we used the double logarithmic plot for two bone dimensions (Moss and Baer, 1956) and specific growth (Ford and Horn, 1959), which is the mean of the bone dimension in logarithms plotted against age on an arithmetic scale. The growth constant, k, from the differential growth equation $y = bx^k$, was calculated from the three point derivative formulae recommended by Ford and Horn (1959). The k is the ratio of the tangents of the specific growth-rate curves of two bone dimensions which are plotted logarithmically against uniform age intervals.

Results

Growth gradients. The specific growth of femur, frontal, nasal, asterion-pterion parietal, and basioccipital of both subspecies are

presented in Fig. 1 and their means and standard errors in Table 1. The figure illustrates that each of the bones of both subspecies grows at a different rate. This is particularly conspicuous where the specific growth curve of one bone crosses that of another. The femur, for example, is one of the shortest bones at birth, but becomes one of the largest at 30 days of age. Similar differences in growth rates occur within the skull. Moss & Baer (1956) demonstrated that the cranial bones in the rat skull tend to grow more rapidly than the facial bones up to a given age, or interphase, when this order is reversed. Although an interphase is not so clearly evident in deermice skulls up to 30 days, there is a definite change in specific growth rates of the cranial or facial skull. The nasal and frontal grow more rapidly than the parietal shortly after birth. On a double logarithmic graph of the nasal plotted against the asterion-pterion parietal (Fig. 2), the increasing specific growth rate of the nasals can be observed at approximately six millimeters, where the curves tend to level. When the growth constant k is calculated from the specific growth rate at each age in the manner suggested by Ford & Horn (1959), the k values (specific growth rate of nasal divided by specific growth rate of parietal) increase until 20 days of age and then decrease to 30 days (Table 2). Although the k values increase to 20 days, they are less than 1.00 only at birth in *gracilis,* which indicates that the nasals grow more rapidly than the parietal from birth. This is unlike the rat skull, in which the early growth of the cranial bones exceeds that of the facial bones shortly after birth (Moss & Baer, 1956).

Subspecies comparisons. Specific growth curves of the nasal and asterion-pterion parietal of both subspecies are compared in Fig. 3a and 3b. *Gracilis* grows at a faster rate than *bairdii,* as indicated by the steeper slope of the *gracilis* curve, which crosses that of *bairdii* between 5 and 10 days of age. The more rapid growth of *gracilis* occurs in most of the bones measured, but some of the curves cross at different ages. Fig. 2 illustrates this change in growth rates of both bones plotted on a double logarithmic graph. The relative growth rates in each subspecies are quite different. Subspecific differences do not resemble the growth of large and small strains of house mice in which the relative rates of growth are constant, but of different magnitude (MacArthur & Chaisson, 1945).

The k values of the nasals and parietals illustrate that the nasals

TABLE 1

MEAN SKULL DIMENSIONS (in mm.), BODY WEIGHT (in grams), AND BRAIN WEIGHT (in mgm) OF *bairdii* (B) AND *gracilis* (G).

Skull Dimension	ssp.	0 Mean±SE (N)	5 Mean±SE (N)	10 Mean±SE (N)	15 Mean±SE (N)
Femur	B	2.78±.200 (4)	4.61±.086 (11)	6.16±.111 (14)	7.95±.112 (15)
length	G	2.58±.056 (12)	4.45±.073 (10)	6.17±.084 (10)	8.35±.113 (13)
Humerus	B	3.10±.100 (4)	4.86±.084 (11)	6.13±.121 (14)	7.41±.125 (15)
length	G	3.15±.074 (15)	4.85±.062 (11)	6.20±.088 (11)	8.16±.092 (13)
Frontal	B	4.43±.160 (5)	6.01±.165 (11)	7.17±.100 (14)	8.38±.121 (15)
length	G	3.77±.091 (14)	5.69±.132 (11)	6.81±.216 (11)	8.57±.142 (13)
Basi-occipital	B	1.97±.084 (4)	2.51±.069 (11)	2.76±.051 (14)	3.02±.038 (14)
mid-sagit.	G	2.03±.039 (14)	2.46±.066 (11)	2.87±.042 (11)	3.33±.080 (12)
Nasal	B	2.86±.087 (4)	4.58±.108 (11)	5.66±.106 (14)	7.00±.119 (15)
length	G	2.78±.053 (15)	4.37±.061 (11)	6.14±.087 (10)	7.30±.095 (13)
Parietal	B	4.05±.206 (5)	5.61±.177 (11)	6.03±.114 (14)	6.50±.161 (15)
(lambda-asterion)	G	3.58±.046 (14)	5.02±.179 (11)	6.36±.140 (11)	7.13±.107 (13)
Parietal	B	3.78±.154 (5)	5.50±.194 (11)	6.30±.087 (14)	7.14±.120 (15)
(asterion-pterion)	G	3.28±.116 (14)	5.32±.077 (11)	6.51±.195 (11)	7.96±.182 (13)
Body wt.	B	1.86±.039 (18)	3.04±.146 (16)	4.86±.153 (17)	6.21±.192 (15)
	G	1.98±.082 (18)	3.18±.148 (16)	4.86±.242 (13)	7.36±.425 (16)
Brain wt.	B	111.1±1.458 (18)	242.2±4.305 (16)	371.5±4.163 (17)	423.7±8.52 (15)
	G	125.8±2.919 (18)	234.1±7.349 (16)	398.9±7.321 (13)	510.8±9.17 (16)

TABLE 1 (*continued*)

MEAN SKULL DIMENSIONS (in mm.), BODY WEIGHT (in grams), AND BRAIN WEIGHT (in mgm) OF *bairdii* (B) AND *gracilis* (G).

Skull Dimension	ssp.	20 Mean±SE (N)	25 Mean±SE (N)	30 Mean±SE (N)
Femur length	B	9.90±.237 (9)	10.04±.161 (15)	11.58±.161 (11)
	G	9.64±.092 (12)	10.59±.273 (12)	12.14±.119 (15)
Humerus length	B	8.95±.101 (9)	8.90±.087 (15)	10.02±.150 (11)
	G	8.78±.089 (11)	9.38±.178 (12)	10.38±.110 (15)
Frontal length	B	9.05±.072 (9)	9.51±.146 (15)	9.35±.116 (11)
	G	9.57±.097 (12)	9.47±.151 (12)	9.72±.115 (15)
Basi-occipital mid-sagit.	B	3.32±.025 (7)	3.22±.041 (15)	3.52±.066 (11)
	G	3.25±.054 (11)	3.48±.053 (12)	3.58±.055 (14)
Nasal length	B	8.13±.259 (9)	8.66±.118 (15)	8.84±.128 (11)
	G	8.19±.113 (12)	8.87±.230 (12)	9.48±.110 (15)
Parietal (lambda-asterion)	B	6.86±.135 (9)	6.91±.118 (15)	7.00±.182 (11)
	G	7.44±.126 (12)	7.29±.100 (12)	7.49±.094 (15)
Parietal (asterion-pterion)	B	7.36±.178 (9)	7.82±.105 (15)	7.83±.188 (11)
	G	8.00±.127 (12)	8.60±.264 (12)	8.55±.158 (15)
Body wt.	B	8.00±.492 (16)	11.12±.218 (6)	11.99±.150 (16)
	G	8.08±.310 (17)	10.34±.619 (9)	10.96±.372 (16)
Brain wt.	B	456.6±10.07 (16)	468.5±11.23 (6)	470.3±4.27 (16)
	G	574.9±7.50 (17)	598.8±12.37 (9)	583.1±7.23 (16)

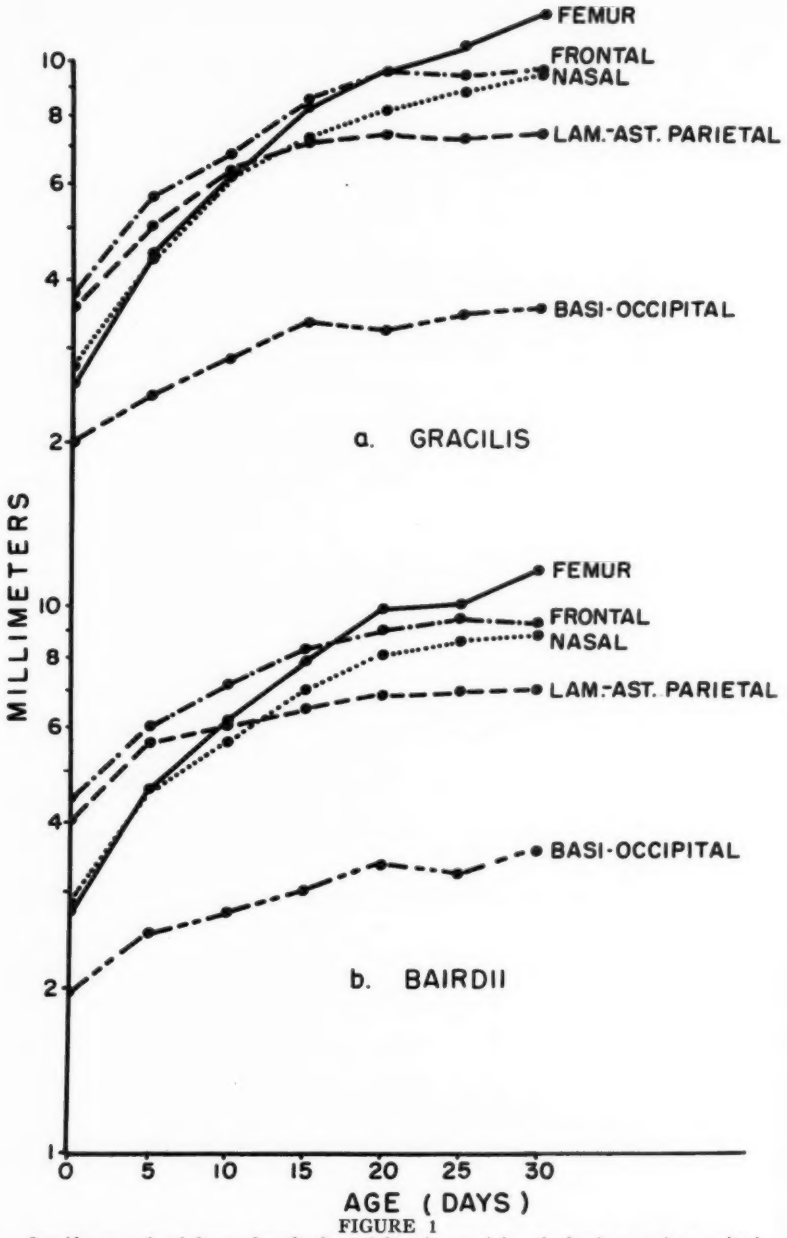

FIGURE 1

Specific growth of femur length, frontal length, nasal length, lamba-asterion parietal, and midsagittal length of the basioccipital in (a) *P. m gracilis* and (b) *P. m. bairdii*.

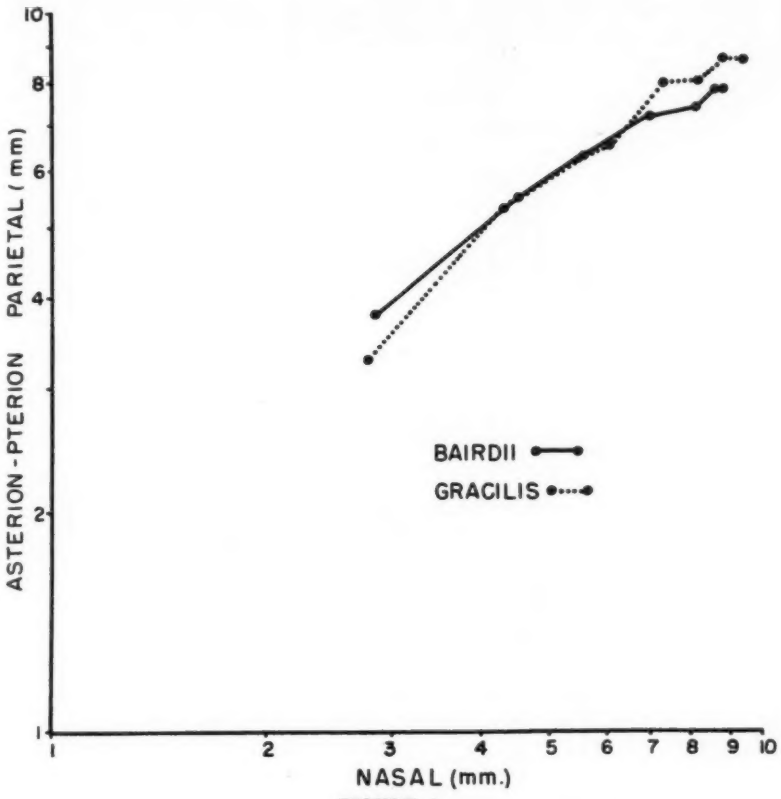

FIGURE 2

Ratio of specific growth rates of nasal length (abscissa) and asterion-pterion parietal (ordinate) of *P. m. bairdii and P. m. gracilis.*

of *bairdii* grow more rapidly in relation to the parietal until 20 days of age (Table 2). At 20 and 25 days of age the nasal growth of *gracilis* in relation to parietal growth then exceeds that of *bairdii.* The facial growth of *bairdii* apparently has begun its accelaration at birth and continues to accelerate until 20 days of age. Facial growth in *gracilis* does not begin to exceed cranial growth until 5 days of age, and then slowly accelerates until 20 days, when there is a growth

TABLE 2

Age in Days	Dimension in mm (natural log.)				Specific Growth Rate					
	Nasal Length		Asterion-pterion Parietal		Nasal		Asterion-pterion Parietal		k	
	Bairdii	Gracilis	Bairdii	Gracilis	Bairdii	Gracilis	Bairdii	Gracilis	Bairdii	Gracilis
0	2.86(1.051)	2.78(1.022)	3.78(1.330)	3.28(1.188)	.120*	.102	.099	.125	1.23	.82
5	4.58(1.522)	4.37(1.475)	5.50(1.705)	5.32(1.671)	.068+	.079	.051	.068	1.34	1.16
10	5.66(1.733)	6.14(1.815)	6.30(1.840)	6.51(1.873)	.042+	.051	.026	.040	1.62	1.27
15	7.00(1.946)	7.30(1.988)	7.14(1.966)	7.96(2.074)	.036+	.029	.016	.021	2.33	1.35
20	8.13(2.096)	8.19(2.103)	7.36(1.996)	8.00(2.079)	.021+	.020	.009	.008	2.34	2.50
25	8.66(2.159)	8.87(2.183)	7.82(2.057)	8.60(2.152)	.008+	.015	.006	.007	0.92	2.18
30	8.84(2.179)	9.48(2.249)	7.83(2.058)	8.55(2.146)	.000±	.012	—.006	—.009	0.00	—1.33

* calculated from $\frac{dy}{dt_0} = \frac{1}{2h}(-3y_0 + 4y_1 - y_2)$, where h = interval in days, y = natural logarithm of dimension (Ford & Horn, 1959)

\+ calculated from $\frac{dy}{dt_1} = \frac{1}{2h}(-y_0 + y_2)$

± calculated from $\frac{dy}{dt_2} = \frac{1}{2h}(+y_0 - 4y_1 + 3y_2)$

spurt in the nasals which persists until 30 days of age. (The negative value of k at 30 days in *gracilis* reflects a sampling error, since each age group comprised a different sample of mice.)

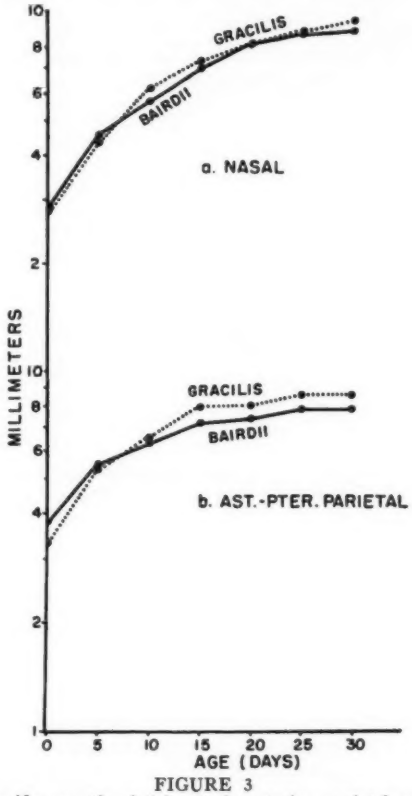

FIGURE 3
Comparison of specific growth of (a) asterion-pterion parietal and (b) nasal length in *P. m. bairdii* and *P. m. gracilis*.

Brain and Body Weight. The specific growth of brain and body are illustrated in Fig. 4. Brain growth in both species is parallel until 10 days of age after which the brain in *bairdii* grows less rapidly than *gracilis*. Both subspecies reach an asymptotic level about 20 days. Between 10 and 20 days of age, brain growth in *gracilis* is faster than

in *bairdii* and reaches a higher level. Increase in body weight is quite similar up to 40 days of age. At 80 days of age, the body weight of *gracilis* is about 14 per cent greater than *bairdii*. The interphase pointed out by Moss and Baer in the rat skull is clearly evident in both subspecies when brain weight is plotted against body weight on a double logarithmic graph (Fig. 5). The interphase, however, occurs much earlier in *bairdii* than it does *gracilis*.

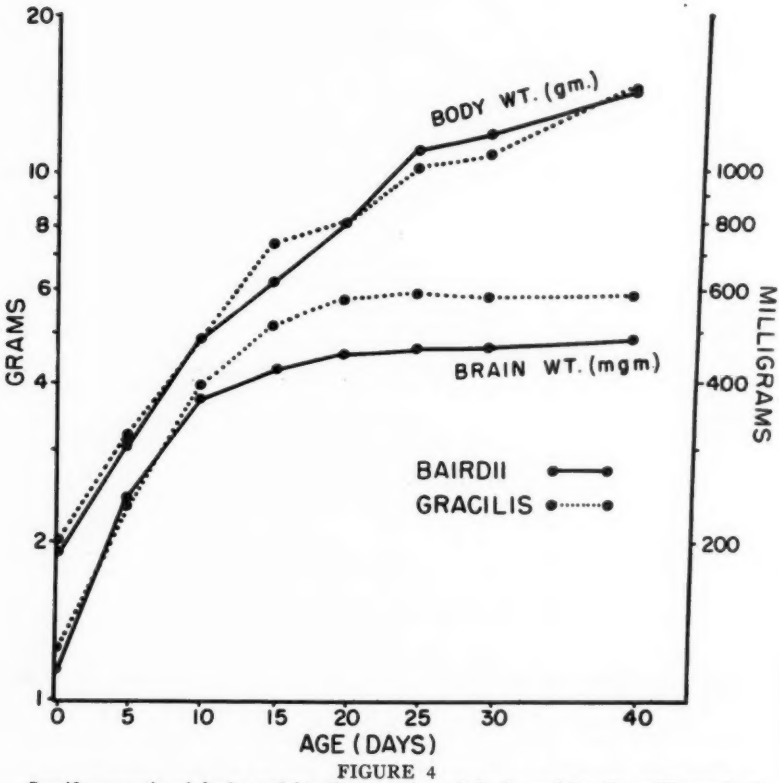

FIGURE 4

Specific growth of body weight (in grams) and brain weight (in milligrams) of *P. m. bairdii* and *P. m. gracilis*.

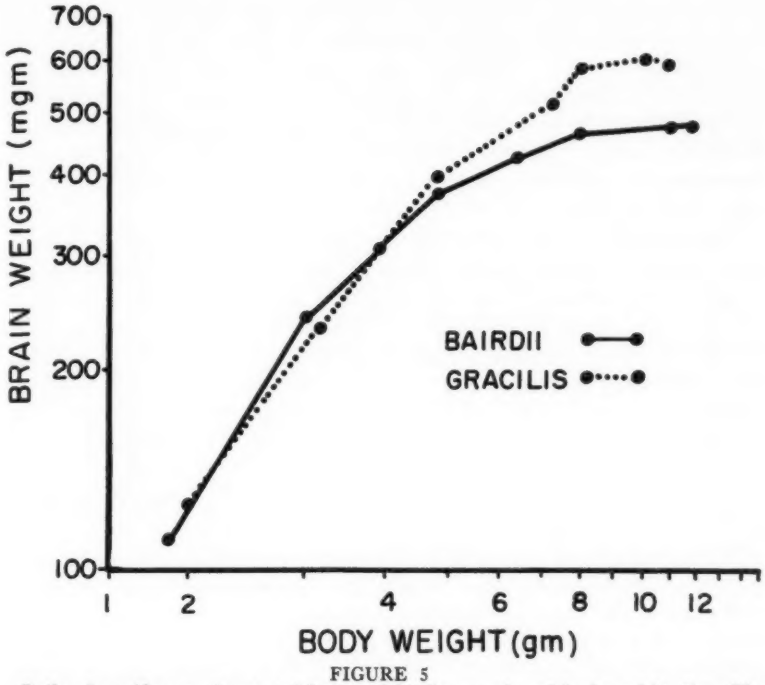

FIGURE 5

Ratio of specific growth rates of body weight (in grams) and brain weight (in milligrams) of *P. m. bairdii* and *P. m. gracilis*.

Discussion

Differences in the body proportions among several species and subspecies of *Peromyscus* may be attributed to general size factors, special size factors, and/or factors determining specific growth rates. After a thorough examination of several species and subspecies of adult *Peromyscus*, Clark (1941) concluded that a general size factor could not account for the differences. He also dismissed the possibility that allometric growth was responsible, although his material was limited to adult specimens. Clark concluded that specific size factors were primarily responsible for the differences in body proportions of *Peromyscus* species. He failed to point out, however, that specific factors may also determine growth rates, which would result in allometric

patterns of growth. In contrast to specific size factors proposed by Clark for differences in body proportions in *Peromyscus,* MacArthur and Chaisson (1945) indicated that a general growth factor was responsible for differences in a large and small strain of domestic mice (*Mus musculus*), but agreed with Clark in discounting the effect of allometry. They concluded that "all the mice collectively form one homogeneous allometric population or tribe," (MacArthur & Chaisson, 1945, p. 309).

The possibility that allometry may contribute to racial differences is indicated in several graphs on the growth of external body measurements of *Peromyscus* from 15 days to maturity by Dice & Bradley (1942). The body length of *P. m. blandus,* for example, increases at a greater rate in relation to tail length than it does in *P. m. bairdii.* This suggestion that allometry is a factor was amplified by McIntosh's (1955) ontogenetic study of *P. m. bairdii* and *P. m. gracilis.* By using a covariance analysis with the femur growth as a constant, McIntosh (1955, p. 50) was able to suggest that "there must be other genetic differences that affect the average rates at which various parts grow relative to one another."

The present study supports McIntosh's conclusion regarding allometric growth patterns and in addition demonstrates that the facial and cranial parts of the skull grow at different rates relative to each other. Each of the two subspecies studied apparently has distinctive allometric growth patterns. During the period of maximum postnatal growth, parietal bones of gracilis grow faster ($k = .82$) or almost as fast ($k = 1.35$) as the nasal bones. At 20 days and after, when the rate of growth decreases, the parietal bones grow much slower, ($k = 2.50$) than the nasal bones. In contrast, the parietal bones of *bairdii* showed a consistently decreasing rate of growth in relation to the nasals until after 20 days of age. This indicates that the cranial part of the skull in *gracilis* grows more rapidly in relation to the facial part than it does in *bairdii,* particularly during the period of rapid postnatal growth, from birth to 20 days. These racial differences in the relative growth of the skull may reflect growth of the underlying neural mass as suggested by Moss & Baer (1956).

When the specific growth of the brains of both races are compared, it can be seen (Fig. 4) that the growth of *gracilis* brains exceeds that of *bairdii* from 10 to 20 days of age. This period roughly corresponds

to the period when facial-cranial growth gradients differ most between *bairdii* and *gracilis*. Although these data do not demonstrate a precise relationship between the growth of the cranial skull and the brain, they do suggest that such a relationship exists. Differential growth of various parts of the brain, such as those examined by Rensch (1958), may demonstrate this relationship more accurately than the weight of the entire brain.

Since different allometric growth patterns are recognizable in the skulls of two subspecies of *Peromyscus maniculatus*, it seems reasonable to expect similar differences to occur in other tissues. The emphasis in this paper was placed on skull growth because it might have relevance to the underlying neural tissue (Moss & Baer, 1956). This is further suggested by different allometry in the brain weights of each subspecies. Further histological and biochemical analysis of the developing brains of these subspecies is necessary to establish allometry within the brain. Although the relationship of differential neural growth to behavior is tenuous, the different rates of development of particular behavioral patterns in each subspecies (King, 1958) suggests a neural basis. After a given behavior has developed sufficiently to be reinforced by the environment, its rate of development may be further increased and channelized into adult forms.

Summary

The skull bones and brain-body weights of *Peromyscus maniculatus gracilis* and *P. m. bairdii* were examined from birth to 30 days of age. Different growth gradients of the facial and cranial skull were demonstrated for each subspecies. The growth of the cranial skull of *gracilis* decreases at a slower rate in relation to the facial skull than it does in *bairdii*. This relatively longer growth period of the cranial skull in *gracilis* corresponds to *gracilis'* greater increase in brain weight from 10 to 20 days of age. It was suggested that differential growth within the nervous system may contribute to the differential rates of development of particular behavioral patterns.

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SPECIFICITY OF A TRANSPLANTABLE TUMOR

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INTRODUCTION

Transplantability of tumors has an interesting history relative to the fields of both genetics and cancer research. With the use of inbred strains of mice early workers were able to formulate some principles of transplantation which elude definition when mice of heterogeneous or unpedigreed stocks are used.

Inasmuch as inbred strains of mice were used for the material advancement of the transplantability of tumors, some small discussion on the production of such strains is in order. Strong and Little (14, 16) must be given the credit for the production of many of the homogeneous lines of mice. Snell (13) developed the so-called isogenic strains, involving the control of one particular gene. Chart I shows the genealogy, in condensed form, of the ancestry of the inbred strains used in this experiment. All of the strains were produced by Strong with the exception of C57BL which was produced by Little and Bagg. The first line on the chart represents unpedigreed mice, i.e. those mice that were derived from unpedigreed origin. The ensuing pedigreed stocks were inbred (brother-sister mating) for twenty generations before they were considered an inbred strain. The high degree of inbreeding produces homogeneity to a degree equivalent to identical twinning in man. With this material the researcher is able to devise experiments which reduce biological variability to a minimum. (It is almost impossible to eliminate completely the variability of living material since alterations, such as mutations, invariably occur from time to time). Thus reproducible results may be obtained.

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Gorer (6), Snell (13) and Amos (1) have been able to apply the findings of transplantability of tissues into the framework of the earlier genetic principles. They developed immunology of transplantation which was referred to as immuno-genetics. By finding the antigens in the blood and correlating them with known genes by linkage, the "histocompatability loci" of transplantation have been partially resolved. Much of this linkage work has been done by Heston (9). In the house mouse the H-2 locus appears to be stronger than other loci in the success or failure of the transplant.

On another front, workers have tried to "adapt" a tumor to grow in mice of other strains that have been naturally resistant to the tumor's growth, (Barrett and Deringer (4) and others). They have found that a tumor in a highly inbred strain of mice grows successfully in a higher percentage of backcross mice after being grown in the F_1 hybrid. Other means, such as injecting foreign materials with the tumor, growing the tumor in chorio-allantoic membranes of the chick, as well as spontaneous changes in the tumor, have been shown to modify the specificity of tumors (8, 10, 11, 20).

In this study an interesting tumor has been used; it varies from the basic concepts of transplant principles, histocompatability and adaptation. This tumor arose in the forestomach of a mouse of the PBR strain after parenteral injection of methylcholanthrene (Bagshaw and Strong, 3). This tumor was first classed as a spindle-cell neoplasm due to the presence of spindle-shaped cells; it has recently been reclassified as an anaplastic carcinoma by Dr. Henry Stoll.* It has been studied rather intensively by Strong (15, 19); Hardy (7, 18); and Zahler (20) at the Biological Station of Roswell Park Memorial Institute. Strong (18) found that the tumor was capable of growing in a number of mice of different inbred strains. Strong and Hardy (18, 7) have not been able to adapt the PBR tumor to grow in mice of the F strain, even after using several methods that bring about adaptation in other tumors.

Procedure and Technique

The mice were under the supervision and care of the authors. Divided wooden boxes with 16 mice to a box were used. The mice

* Henry C. Stoll, M. D., Associate Chief Cancer Research Pathologist, Roswell Park Memorial Institute, Buffalo, N. Y. Present Address: V.A. Hospital, Brooklyn, N.Y. (Personal communication to Dr. L. C. Strong).

were obtained at weaning age from the various staff members of the Biological Station. Sawdust and cotton were provided for nesting material, while Shamel laboratory mix and water were made available *ad libitum*. The boxes were changed weekly, at which time whole mixed grains, (wheat, oats, sunflower seeds and calf meal pellets) treated with cod-liver oil were provided.

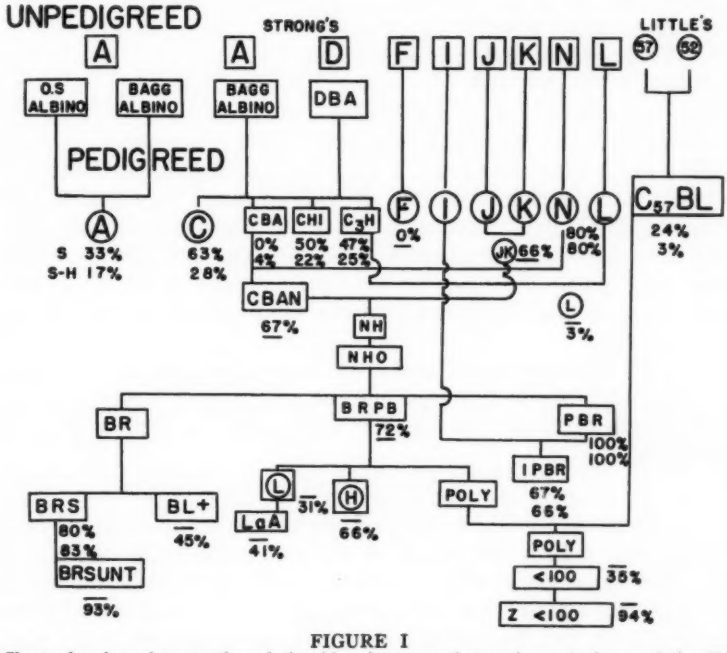


FIGURE I

Chart showing the genetic relationships between the various stocks used in this investigation. The small percentage figures show the percentage susceptibility in two separate experiments. The symbol S indicates the experiment of Strong; S-H, Snethen and Hicks.

Nineteen strains of mice were used, with a total number of 626 mice. The strains tested are shown on the Genealogy Chart I. The mice were divided into two nearly duplicate series, into which the tumor was subcutaneously transplanted. Twenty-two transplants were performed on PBR mice prior to the initiation of the experiment. The

balance of the PBR mice were used as controls, when transplants were made into the various strains.

The strains have been divided as follows:

1. Independent to PBR: A, C, CHI, C3H, I, L, C57BL. Note: C, CHI, and C3H, collateral to CBA, have been classified as independent to PBR because of the introduction of so much new genetic material from both hybridization and possibly mutation.
2. Ancestral to PBR: CBA, N.
3. Collateral to PBR: BRS, BRSUNT, Bl+, L (low), LaA, H (high), <100, $2 < 100$.
4. Derivative of PBR: IPBR.

Tumor 75B (an anaplastic carcinoma) from the strain of origin (PBR) was used in this study. A trocar was used to implant the piece of tumor into the right flanks of the various mice; only pearly white tissue was implanted. Samples of tumor tissue were taken for preparation of slides for histological examinations. The mice were palpated for "takes" every seven days for a period of 28 days. Any tumor or mass that could be felt at the site of implantation was read as a positive.

Since the mice were sexed, and gross observation indicated a possible sex difference, the final results on the twenty-eighth day following implantation of tumor were also read by sexes as well as by total population.

Observations

The tumor, as would be expected, grew well in the strain of origin (PBR) with a high percentage of "takes" palpable by the seventh day. The tumor grew so rapidly, between the fourteenth and twenty-first day, that after one or two deaths, the rest of the PBR mice were destroyed. In the other strains varying rates of growth of the tumor were observed (Table I). Most of the collateral strains showed high tolerance for the initial growth period of approximately fourteen days, after which the tumors began to show signs of regression. However, contrary to expected results, most of the strains tested showed some degree of susceptibility by carrying the tumor on to the close of the experimental period. From the table and percentage of "takes" recorded by Strong (19) on the Genealogy Chart these new 14-day results corroborate Strong's earlier observations. From the final data it is interesting to note that strains I, L (low, C57BL, and CBA have

TABLE I
SUSCEPTIBILITY AND GROWTH OF AN ANAPLASTIC CARCINOMA IN MICE OF VARIOUS STRAINS

	No. of Mice	7 Days	14 Days	21 Days	28 Days
Control					
PBR	66	73%	100%	100%	100%
Independent					
A	30	27	33	17	17
C	32	25	53	28	28
CHI	32	59	59	38	22
C3H	48	38	67	42	25
I	40	8	25	15	11
L	36	56	68	17	3
C57BL	58	45	35	9	3
Ancestral					
CBA	24	54	21	8	4
N	43	47	86	79	84
Collateral					
BRS	41	59	85	83	83
BRSUNT	15	73	93	93	93
B1+	33	49	70	58	45
L (low)	32	34	44	34	31
LaA	27	37	63	56	41
H (high)	29	45	79	69	66
<100	18	17	50	33	33
2<100	15	69	94	93	93
Derivative					
IPBR	27	94	93	78	66

approximately the same modal percentage of "takes". The first three strains are unrelated to the PBR strain, but the CBA is ancestral. In the collateral strains of CBA we find approximately a 25 percent take in C, CHI, and C3H—a unique situation inasmuch as Amos (2) has found the histocompatibility of the H-2 locus of these four strains to be as follows: C/St with H-2q, while C3H, CHI, and CBA are H2k. In the collateral strains to PBR, a fairly high to a very high susceptibility to the transplant was observed with the exception of strain H (high) and strain <100. The <100 and 2 < 100 being sublines of the Poly mice of Strong are closely related to each other by common descent. However, the mice show a great degree of variability: 33 per cent as compared to 94 percent respectively. In the <100 strain the piebald mice showed a greater degree of susceptibility than the self color phase of the same strain.

The graphs (Charts II, III, IV) show the development and regression of the tumor in the various strains of mice. Regression in all strains except the B1+ was by internal absorption. In the B1+, regres-

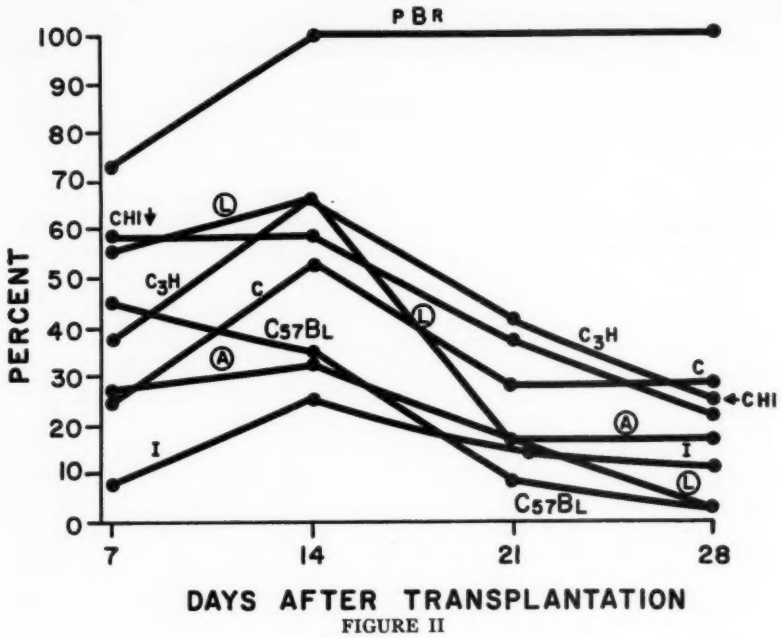


FIGURE II

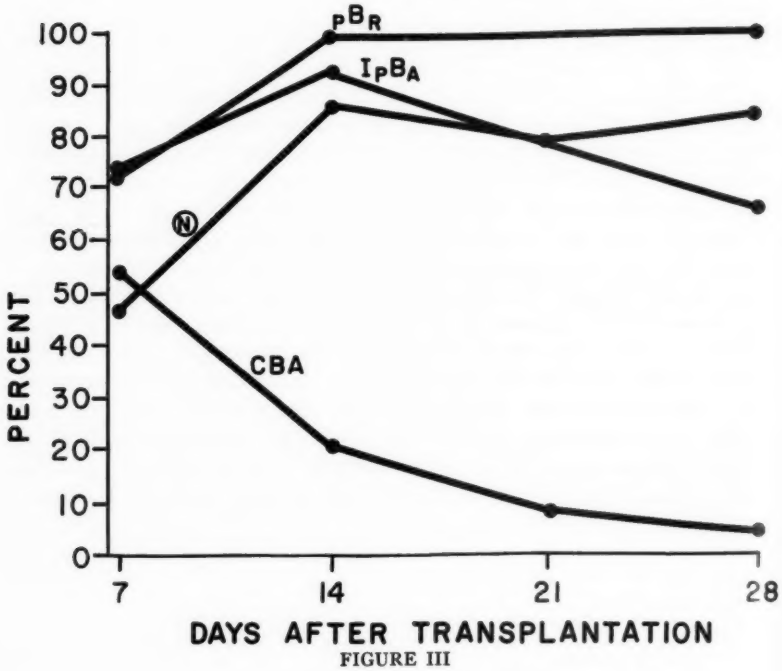
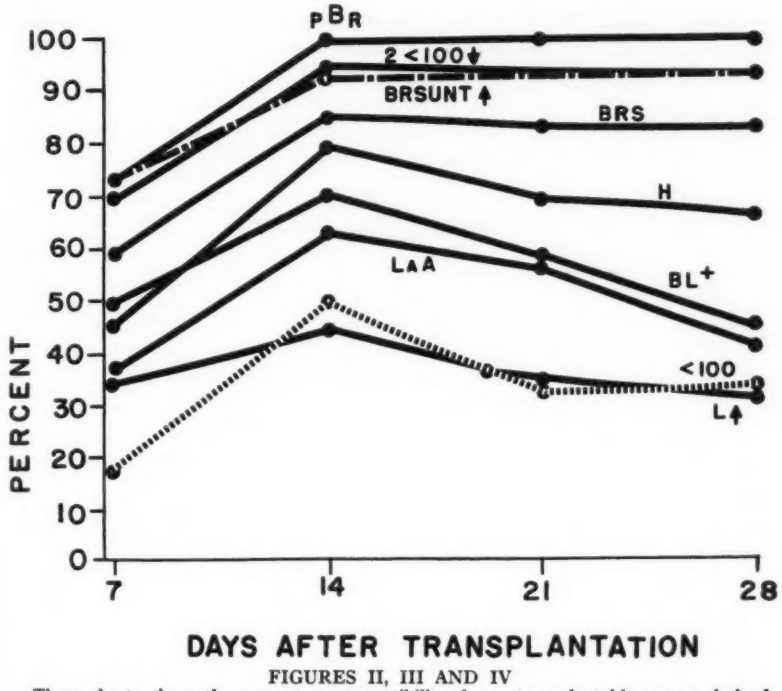


FIGURE III



FIGURES II, III AND IV

These charts show the percentage susceptibility for a transplantable tumor derived from PBR source growing in mice of the various strains for the first 28 days following the grafting of the tumor.

sion occurred as a result of ulceration of the incompatible tumor tissue through the skin.

Some possible sex difference may exist in the growth of the tumor in mice of strains N, L (low), IPBR, and H (high). (Table II).

Discussion

From our observations, it appears that this tumor continues to behave as an exception to the general rules of transplantability. Strong's histological examination of the tumor material used in our experiment indicates that structurally the tumor has not changed, (Bagshaw & Strong, 3). The specificity of the tumor seems to be the

same as when it was first tested rather than less specific after a large number of serial transplants as sometimes occurs in other transplanted tumors. Since the scope of the experiment was limited, our data do not reveal any new specific conclusions. However, the peculiarities and permanence of the tumor material make it an extremely important tool for further research studies.

TABLE II

COMPARISON OF THE POSITIVE TAKES IN MALE AND FEMALE MICE FOUR WEEKS AFTER TRANSPLANTATION

	Male		*Female*	
	Total Number	Per cent Positive	Total Number	Per cent Positive
Independent				
A	16	13	14	21
C	16	25	16	31
CHI	16	13	16	31
C3H	22	23	28	29
I	18	6	22	14
L	15	0	22	5
C57BL	28	4	30	3
Ancestral				
CBA	14	7	10	0
N	21	71	22	91
Collateral				
BRS	20	85	21	81
BRSUNT	12	92	3	100
B1+	16	50	17	53
L (low)	16	19	16	44
LaA	10	50	17	45
H (high)	13	73	16	63
<100	6	33	12	33
2<100	6	83	9	100
Derivative				
IPBR	11	73	16	63

The genetic relationships of mice of the collateral strains of CBA, C, CHI, and C3H respectively can be determined possibly by using mice which are hybridized from collateral strains and the PBR strain. The growth of the tumor could then be tested in the F_2 and backcross to the C, CHI, CBA and C3H lines. These tumor transplants might very well be more fruitful than those using the F strain, which has peculiarities* as far as mice are concerned. In personal talks with Dr. Amos, the authors found that histocompatibility of the tumor in the collateral strains and the hosts could not be explained on the

* Histocompatibility as tested by Dr. Amos does not follow patterns of other inbred strains.

basis of the H-2 locus. Immunological studies concurrent with the hybridization and backcross generations, however, would be of value in trying to establish a histocompatibility factor or factors.

Barrett and Deringer's (4) work on adaptation could also be applied to CBA as a resistant ancestral strain. Since this tumor could not be adapted by Strong and Hardy (18) in the F strain, the development of the tumor in the resistant strain after a transient growth in the F_1 of the CBA x PBR would confirm the hypothesis that this adaptation may be possible in only genetically related mice. Failure of the tumor to grow would prove that the tumor cannot be adapted by this procedure.

The variance of susceptibility in the Poly sublines leads to the development of an extended genetic test using the 75B tumor on all four strains, to reveal the degree of susceptibility in large numbers of mice of each subline. The sex difference lends itself to still another study using the N, L (low), IPBR, H (high) strains of mice.

No mention of virus contamination has been made; but the studies of Eddy and Steward (5) and Mirand (11) indicate that virological work must be done to eliminate this possibility for an explanation of the peculiarities of this work.

Summary

In the nineteen strains of mice used in the present experiment, the 75B tumor (an anaplastic carcinoma) grew to varying degrees in mice of all strains. This characterizes the PBR transplantable tumor as an unusual one. The tumor appears to retain its original specificity as found by Strong (5).

The collateral strain of CBA (one of the ancestral strains to PBR, the strain of origin of the tumor) show a higher susceptibility to tumor growth than that of the immediate parental stock (the PBR).

The regression of the transplantable tumor in mice of all strains except the Bl+ was by internal absorption; in mice of the Bl+ strain the regression occurred as an ulceration through the skin.

Based on gross observation of a small number of animals a sex difference in the growth of the tumor seems to exist in mice of strains N, L (low), IPBR, and H (high).

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